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# INFLUENCE OF ELECTROLYTES UPON THE VISCOSITY OF DOUGH.

By L. J. HENDERSON, W. O. FENN,* and EDWIN J. COHN.*

*(From the Wolcott Gibbs Memorial Laboratory, Harvard University, in Cooperation with the Division of Food and Nutrition, Medical Department, U. S. Army.)*

(Received for publication, December 6, 1918.)

The rising of bread and the quality of the baked loaf depend upon the nature of the dough and especially upon such of its properties as tenacity, ductility, and elasticity. These properties are hard to define theoretically in this complex colloidal system, but they are, as we have found, related to viscosity or to whatever may determine the resistance of dough to stirring and to flowing through a tube. Accordingly, experiments have been made to measure what may provisionally be called the viscosity of dough under conditions which reveal the influence of the hydrogen ion concentration and of a variety of electrolytes upon this property. The result of the experiments is proof that in addition to the effect of water content and of the presence of substitutes lacking gluten, the viscosity of dough is much influenced by these two factors. Certain important points in baking practice are explained by these results.

## *Measurement of the Viscosity of Dough.*

The property that we shall call viscosity is quantitatively measured in the experiments reported by the resistance of dough to stirring. 12 gm. of dough are packed in the torsion viscosimeter represented, full size, in Fig. 1.

The viscosimeter is placed in a thermostat at 30°C. and the dough allowed to stand 10 minutes in order to bring it to the temperature of

* Lieutenant, Sanitary Corps, U. S. Army.

the bath. The paddles are forced through the dough by the fall of 150 gm. through 2 meters. The rate of the falling of the weight is recorded and measures the viscosity of the dough.

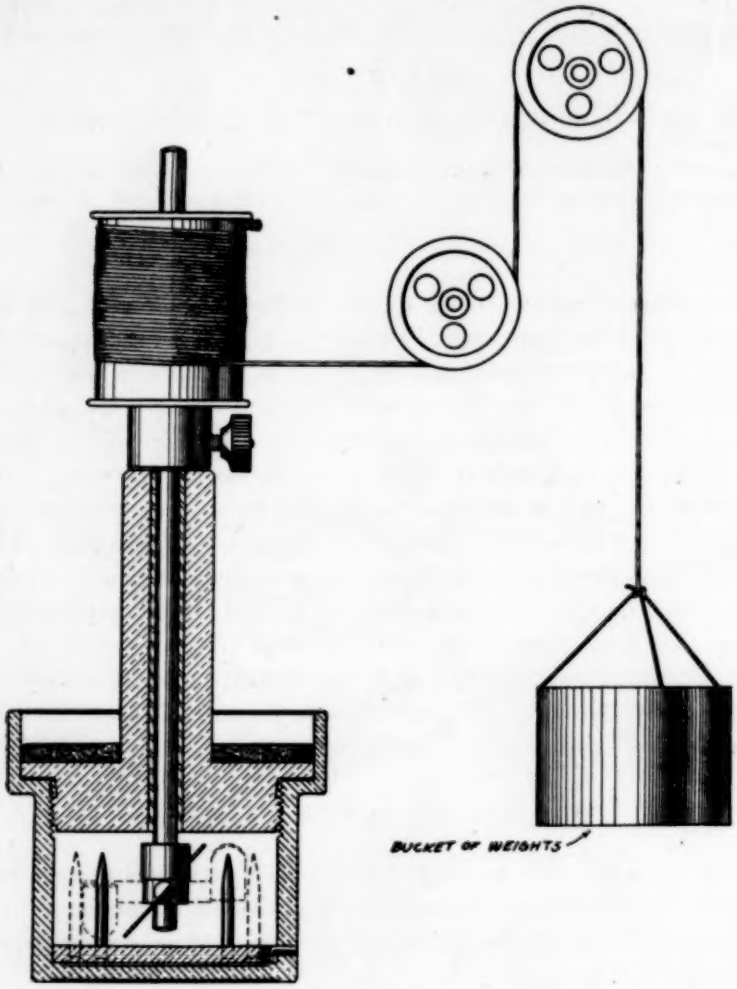



FIG. 1.

The turning of the dough inside the viscosimeter is prevented by four equidistant pins. It has been found convenient to attach the pins to a plate rather than to the bottom of the viscosimeter to facili-

TABLE I.

*Viscosity of Dough at Different Hydrogen Ion Concentrations. Time in Minutes of Fall of Weight in Torsion Viscosimeter.*

| Estimated pH of dough. | Mols of HCl per 1,000 gm. of flour. | Experiment 725. 1,000 gm. of Flour A, 600 cc. $H_2O$. | Experiment 664. 1,000 gm. of Flour B, 590 cc. $H_2O$. | Experiment 728. 1,000 gm. of Flour D, 580 cc. $H_2O$. | Experiment 747. 1,000 gm. of Flour M, 540 cc. $H_2O$. |
|---|---|---|---|---|---|
| | | *min.* | *min.* | *min.* | *min.* |
| 3.65 | 0.054 | | | | 15.8 |
| 3.80 | 0.048 | 16.2<br>16.4 | | | |
| 3.85 | 0.046 | | | 15.7<br>14.0 | |
| 3.90 | 0.043 | | | | 12.8<br>11.8 |
| 4.05 | 0.040 | | 14.0<br>12.5 | | |
| 4.20 | 0.036 | 15.0<br>12.9<br>14.5 | | | |
| 4.20 | 0.035 | | | 8.3<br>11.3<br>10.1 | |
| 4.30 | 0.032 | | | | 11.1<br>10.7 |
| 4.40 | 0.030 | | 10.6<br>12.3 | | |
| 4.65 | 0.024 | 13.9<br>14.2<br>12.1 | | | |
| 4.70 | 0.023 | | | 8.4<br>10.1<br>9.6 | |
| 4.75 | 0.022 | | | | 10.1<br>9.6 |
| 4.85 | 0.020 | | 10.0<br>9.2 | | |
| 5.20 | 0.012 | 14.2<br>17.2 | | | |

TABLE I—*Concluded.*

| Estimated pH of dough. | Mols of HCl per 1,000 gm. of flour. | Experiment 725. 1,000 gm. of Flour A, 600 cc. $H_2O$. | Experiment 664. 1,000 gm. of Flour B, 590 cc. $H_2O$. | Experiment 728. 1,000 gm. of Flour D, 580 cc. $H_2O$. | Experiment 747. 1,000 gm. of Flour M, 540 cc. $H_2O$. |
|---|---|---|---|---|---|
| | | *min.* | *min.* | *min.* | *min.* |
| 5.20 | 0.012 | | | 10.0<br>11.0 | |
| 5.22 | 0.011 | | | | 11.9<br>11.6 |
| 5.25 | 0.010 | | 13.7<br>11.0 | | |
| 5.50 | 0.005 | | 12.7<br>14.3 | | |
| 5.80 | 0 | 20.0<br>20.2 | 18.1<br>14.3 | 15.5 | 15.2 |
| | NaOH | | | | |
| 6.50 | 0.01 | | 19.4<br>24.1 | | |
| 7.27 | 0.02 | | 31.0<br>25.0 | | |
| 7.62 | 0.03 | | 40.2 | | |

tate the cleaning of the apparatus. A small pin keeps the plate from turning.

The flour is best gathered together with a stirring rod in the beaker into which the various solutions are run. The volume of water, including that containing dissolved electrolytes, added to the flour is recorded in cc. of $H_2O$ per 1,000 gm. of flour. The quantity of electrolytes is recorded in mols per 1,000 gm. of flour. The small size of the doughs used in these experiments made it necessary further to work them by hand. This was always done by the same experimenter and for precisely 2 minutes. The solutions of electrolytes of different strength were added with the water.

No great accuracy is possible in carrying out such measurements, first, because a very exact control of the water content is almost impossible without undue expenditure of time, and secondly, because slight differences in the packing of the viscosimeter are not without serious influence. However, the data will show the degree of accuracy

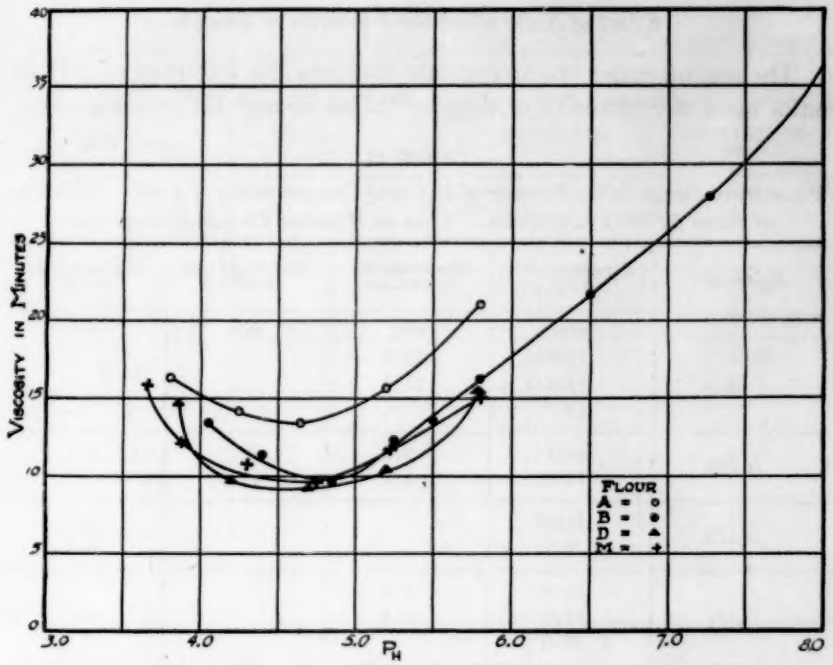



FIG. 2.

of the observations and, as we believe, justify such use of them in drawing conclusions as is at present necessary.

*Effect of Hydrogen Ion Concentration upon the Viscosity of Dough.*

Our first series of experiments concerns the influence of hydrogen ion concentration upon the viscosity of dough prepared from four different flours. Differences of reaction were produced by the addition of solutions of hydrochloric acid and sodium hydroxide and estimated with the help of the concentration cell. The varied character of the four flours is indicated by the difference in the water content of their approximately isoviscous doughs.

The results clearly show that a single measurement of viscosity possesses no great significance (Table I and Fig. 2). Nevertheless it is evident that with variation in hydrogen ion concentration viscosity passes through a minimum a little on the acid side of pH 5. Jessen-Hansen[1] has found pH 5 to be the best hydrogen ion concentration in bread making and it is also, as we have observed, the most favorable reaction for the rising of dough.

[1] Jessen-Hansen, H., *Compt. rend. trav. Lab. Carlsberg*, 1911, v, no. 10.

### *Effect of Salts upon the Viscosity of Dough.*

The second series of experiments concerns the influence of certain salts upon the viscosity of dough (Tables II and III and Fig. 3)

TABLE II.

*Viscosity of Dough in the Presence of Different Concentrations of Salts. 1,000 Gm. of Flour B; 590 Cc. of Water. Time in Minutes; Torsion Viscosimeter.*

| Mols of salt. | Experiment 671. NaCl | Experiment 684. Na lactate.* | Experiment 680. $Na_2SO_4$ | Experiment 680. $MgSO_4$ |
|---|---|---|---|---|
| | *min.* | *min.* | *min.* | *min.* |
| 0 | 13.7<br>13.4<br>15.7 | 12.4<br>11.3 | | |
| 0.059 | 10.8<br>11.4 | 9.2<br>8.9 | 10.7<br>11.2 | 8.6 |
| 0.118 | 11.0<br>9.7 | | | 6.9<br>6.8 |
| 0.177 | 13.9<br>11.0<br>10.0 | 7.6<br>8.4 | | |
| 0.194 | | | 6.6<br>6.7 | |
| 0.236 | | | | 4.3<br>3.7 |
| 0.295 | 13.4<br>13.3 | | 4.5<br>5.0 | |
| 0.354 | | 5.9<br>7.0 | | 3.0<br>3.5 |
| 0.590 | 13.9<br>18.9<br>13.9 | 7.6<br>7.6 | | 4.9<br>4.4 |

* The moisture of the flour slightly increased the water content and decreased the viscosity of the doughs in Experiment 684. This is shown by the viscosity of dough containing no Na lactate. A mixture of 49 parts of Na lactate and 1 part of lactic acid approximately isohydric with flour was used.

These experiments show that the addition of a small amount of salt to dough tends to diminish viscosity. As the concentration of the salt increases, however, there is commonly, and probably with

TABLE III.

*Viscosity of Dough in the Presence of Different Concentrations of Salts. 1,000 Gm. of Flour M; 550 Cc. of Water. Time in Minutes; Torsion Viscosimeter.*

| Mols of salt. | Experiment 744. $KBrO_3$ | Experiment 742. $NH_4Cl$ | Experiment 750. $MgCl_2$ |
|---|---|---|---|
| | min. | min. | min. |
| 0 | 14.2 | 12.1<br>12.7 | |
| 0.064 | 11.9<br>10.5 | 11.4<br>12.3 | 12.6<br>11.7 |
| 0.137 | 11.0<br>13.0 | 10.0<br>11.4 | 11.2<br>10.8 |
| 0.275 | 11.8<br>10.4 | 10.4<br>13.7<br>13.7 | 10.7<br>11.8 |
| 0.550 | | 14.4<br>15.6 | 18.5<br>18.7 |

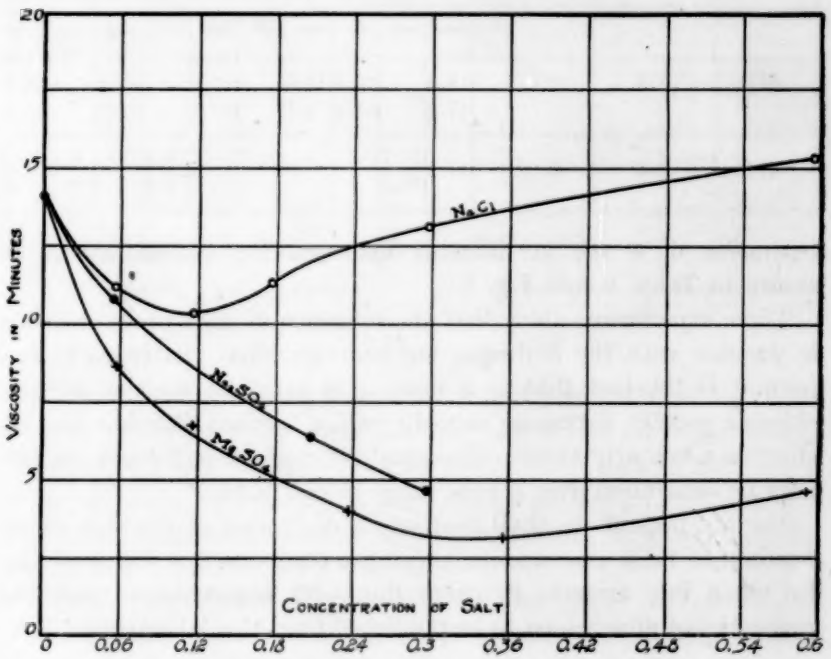



FIG. 3.

sufficiently high concentration always, a later rise in viscosity. Among the salts which we have studied the sulfates of sodium and magnesium have the most marked effect. In these cases it is sufficient to handle the dough in order to note a certain "slackness" or diminished firmness.

### *Effect of Salts upon the Viscosity of Dough at Different Hydrogen Ion Concentrations.*

The third series of experiments concerns the simultaneous influence of acid or alkali and salt upon the viscosity of dough. The effect of different concentrations of a salt at two hydrogen ion concentrations is illustrated in Table IV and Fig. 4; the effect of the same concentration of a salt at different hydrogen ion concentrations is shown in Table V and Fig. 5.

TABLE IV.

*Viscosity of Dough in the Presence of Different Concentrations of NaCl at Different Hydrogen Ion Concentrations. (Time in Minutes.)*

| Experiment No. | Estimated pH of dough. | Mols of HCl per 1,000 gm. of flour. | 1,000 gm. of Flour B; 590 cc. $H_2O$. Mols of NaCl. | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 0 | 0.049 | 0.059 | 0.118 | 0.147 | 0.177 | 0.246 | 0.295 | 0.492 | 0.590 |
| | | | *min.* | *min.* | *min.* | *min.* | *min.* | *min.* | *min.* | *min.* | *min.* | *min.* |
| 671 | 5.8 | 0.0 | 13.7 | | | | | 13.9 | | | | 13.9 |
| | | | 13.4 | | 10.8 | 11.0 | | 11.0 | | 13.4 | | 18.9 |
| | | | 15.7 | | 11.4 | 9.7 | | 10.0 | | 13.3 | | 13.9 |
| 673 | 4.8 | 0.02 | 13.3 | 11.7 | | | 8.7 | | 9.1 | | 8.7 | |
| | | | | 10.4 | | | | | 9.4 | | 9.5 | |

These experiments show that the influence of salts upon viscosity is variable with the hydrogen ion concentration. Particularly important is the fact that at a more acid range of reaction sodium chloride greatly depresses viscosity while calcium chloride has its effect in a less acid range. The great effect of the sulfates, however, may be recognized over a wide range of reaction.

For the present we shall postpone a discussion of the theoretical bearings of these experiments, which are often obscure, and may best be taken into account in connection with observations upon the properties of gluten soon to be published from this laboratory.

The importance of these results in bread making depends upon their relation to a variety of other facts. First, it has long been known that bread possesses a distinctly acid reaction. This is due to the fact that beginning with an acid dough of hydrogen ion concentration $10^{-6}$ N or more, the process of fermentation produces an augmentation of acidity. The observations of Jessen-Hansen in the Carlsberg Laboratory confirm this fact by quantitative measurements

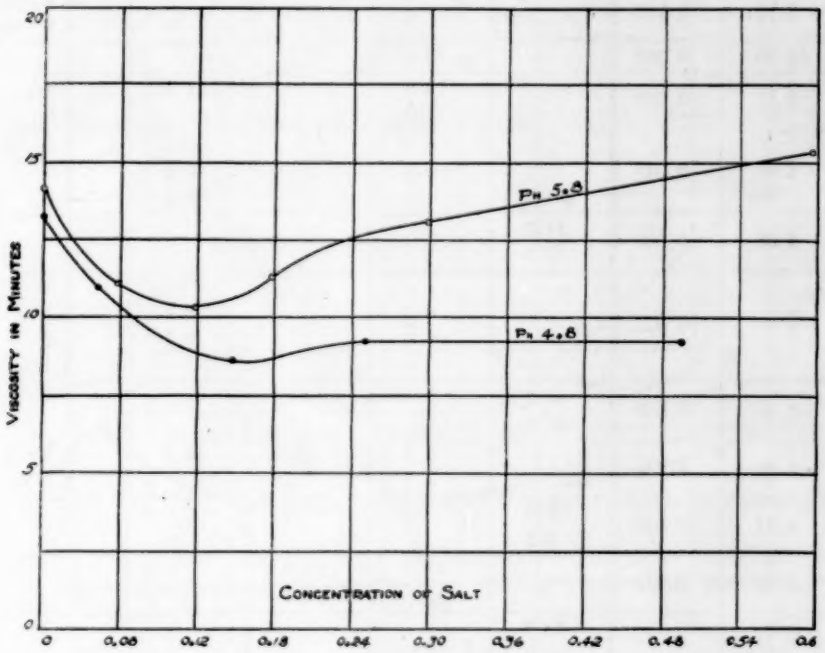



FIG. 4.

and lead their author to the conclusion that about $10^{-5}$ N hydrogen ion concentration is the most favorable for bread making. Observations of our own (except with a very weak flour) agree with those of Jessen-Hansen. We have also made numerous measurements of the hydrogen ion concentration of bread procured in the market, which are entirely consistent with this view.

Second, our studies reported in another paper of the rising of dough and the carbonic acid production during fermentation

TABLE V.

*Viscosity of Dough in the Presence of Salts at Different Hydrogen Ion Concentrations. Time in Minutes; Torsion Viscosimeter.*

| Estimated pH of dough. | Mols of HCl per 1,000 gm. of flour. | Experiment 664. 1,000 gm. of Flour B. 590 cc. $H_2O$. | Experiment 672. 1,000 gm. of Flour B. 590 cc. $H_2O$. NaCl 0.59 mols. | Experiment 677. 1,000 gm. of Flour B. 590 cc. $H_2O$. $CaCl_2$ 0.393 mols. | Experiment 683. 1,000 gm. of Flour B. 590 cc. $H_2O$. NaCl 0.314 + $CaCl_2$ 0.079 mols. | Experiment 681. 1,000 gm. of Flour B. 590 cc. $H_2O$. $Na_2SO_4$ 0.196 mols. |
|---|---|---|---|---|---|---|
| 2.70 | 0.118 | | | | | 12.4<br>8.8 |
| 3.00 | 0.088 | | 16.4 | | | |
| 3.55 | 0.059 | | 12.8<br>11.0 | | 17.3 | 5.4<br>6.0 |
| 3.85 | 0.047 | | 6.3<br>5.8 | | 10.3<br>7.6<br>10.3 | 5.2<br>5.2<br>4.5 |
| 4.05 | 0.040 | 14.0<br>12.5 | | | | |
| 4.25 | 0.035 | | 8.3<br>7.4 | | 8.7<br>9.2 | 4.5<br>5.3<br>6.2<br>4.4 |
| 4.40 | 0.030 | 10.6<br>12.3 | | | | |
| 4.65 | 0.024 | | 9.8<br>8.3 | 16.9 | 10.2 | 4.9<br>4.7 |
| 4.85 | 0.020 | 10.0<br>9.2 | | | | |
| 5.20 | 0.012 | | 13.7<br>12.5 | 14.9<br>14.6 | | |
| 5.25 | 0.010 | 13.7<br>11.0 | | | | |
| 5.50 | 0.006 | | | 14.2<br>13.2 | 13.3<br>13.2 | |
| 5.55 | 0.005 | 12.7<br>14.3 | | | | |
| 5.80 | 0 | 18.1<br>14.3 | 18.3<br>14.8 | 11.8<br>13.4 | | 6.7<br>6.6 |
| 6.50 | NaOH<br>0.010 | 19.4<br>24.1 | | | | |
| 6.60 | 0.012 | | 17.1<br>16.4 | 15.5<br>15.9 | | |
| 7.27 | 0.020 | 31.0<br>25.0 | | | | |
| 7.70 | 0.024 | | | | | 12.7 |
| 8.20 | 0.030 | 40.2 | | | | |

indicate that the best results are obtained near the point of minimum viscosity.

Finally, the favorable results in the baking industry obtained by the use of calcium salts in the absence of acid and of acid in the absence of calcium salts also indicate that it is advantageous to employ dough at a relatively low viscosity, or, speaking more correctly, to

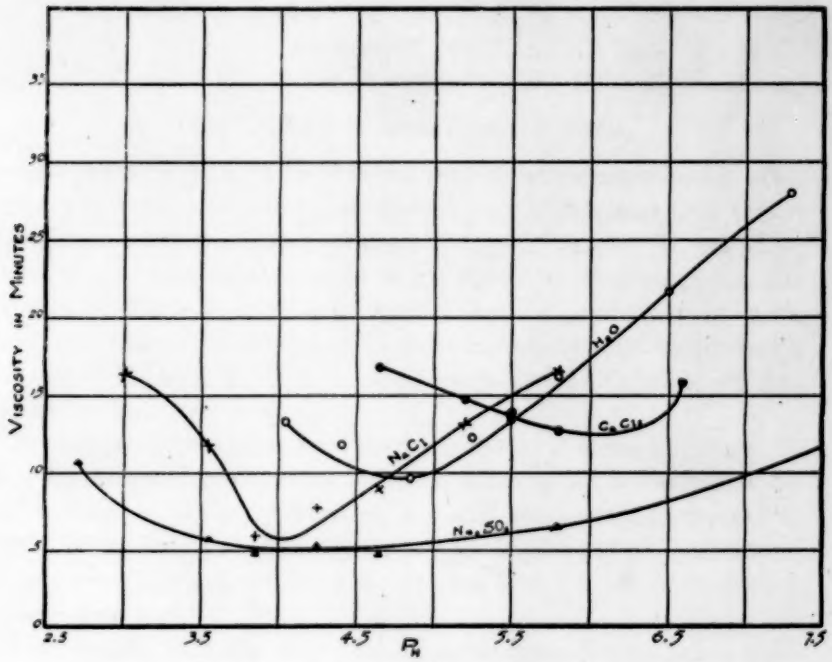



FIG. 5.

reduce whatever quantity of gluten may be present to a condition in which its viscosity is low.

As far as our investigations of this and related questions have proceeded, they indicate that in bread making the action of acids, bases, and salts, perhaps with the exception of potassium bromate, is favorable chiefly through the effect upon viscosity. Any influence upon the activity of yeast appears to be a matter of secondary importance.

# COMPARATIVE STUDIES ON RESPIRATION.

## VI. Increased Production of Carbon Dioxide Accompanied by Decrease of Acidity.

By MARIAN IRWIN.

(*From the Laboratory of Plant Physiology, Harvard University, Cambridge.*)

(Received for publication, January 9, 1919.)

In a previous paper[1] I have shown that high concentrations of ether greatly increase the production of $CO_2$ by animals and that this is associated with irreversible changes ending in death. It might be thought that this increase could be explained on the ground that the process of death is often accompanied by the production of lactic acid, and this might displace $CO_2$ from carbonates and bicarbonates present in the tissues. The resulting evolution of $CO_2$ might be mistaken for increased respiration.

In order to obtain some light on this question it seemed desirable to experiment upon an organism in which death is not accompanied by an increase in acidity. Suitable material for such experiments is furnished by the petals of many flowers containing natural indicators, whose colors show that during the process of death the cell contents become less acid.[2]

Two species of *Salvia* were used in these experiments, *Salvia involucrata* and *Salvia splendens*. The natural indicators contained in the petals were calibrated by placing the petals in boiling water and then transferring them to buffer solutions of known pH value. They remained in the buffer solutions until complete penetration had taken place (this required only a few minutes). As the color of the indicator in various buffer solutions was known, it was a simple matter to determine the changes in acidity[3] which take place in flowers immersed

[1] Irwin, M., *J. Gen. Physiol.*, 1918–19, i, 209.

[2] Haas, A. R., *J. Biol. Chem.*, 1916, xxvii, 233.

[3] The petals contain some cells which lack the indicator; their acidity was consequently not determined.

in a 7.3 per cent (by volume) solution of ether. These changes are shown in Table I.

The $CO_2$ output of *Salvia involucrata* was measured by the methods used in the previous investigation. The petals[4] were immersed in tap water and the normal respiration was determined. The petals were then immersed in a solution of 7.3 per cent ether (by volume) and new determinations were made. The results are shown in Fig. 1.

Objection might be made to submerging the petals in liquid on the ground that this condition is abnormal and that gas might be given off more rapidly through the stomata when ether is present (as the result of changes in surface tension). For this reason it seemed de-

TABLE I.

*Changes in the pH Value of Salvia Petals during the Process of Death Caused by Immersion in Solution of 7.3 Per Cent Ether (by Volume).*

| Length of exposure to ether. | pH value. | |
|---|---|---|
| | *Salvia involucrata.* | *Salvia splendens.* |
| min. | | |
| 0 | 4.5 | 3 |
| 3 | 5.0 | |
| 5 | 6.0 | |
| 6.5 | | 5 |
| 7 | 7 to 8 | |
| 10 | | 7 to 8 |

sirable to repeat the experiments by the method described by Osterhout.[5] As flowers of *Salvia involucrata* were no longer available *Salvia splendens* was used. The corollas were placed in a glass tube, the normal respiration was measured, and sufficient ether was then introduced into the apparatus to saturate the air with ether vapor at 20°C.[6] The rate of respiration was then determined at frequent intervals. In respect to the per cent of increase produced by ether the results agreed closely with those shown in Fig. 1.

[4] In all the experiments the entire corolla was used, care being taken to detach it with as little injury as possible.

[5] Osterhout, W. J. V., *J. Gen. Physiol.*, 1918–19, i, 17.

[6] The petals were at no time in contact with liquid.

Experiments were made to determine whether exposure to ether causes an increase in the consumption of oxygen. For this purpose the apparatus shown in Fig. 2 was employed. The petals were placed in a glass tube A, which was connected at one end (by tubes of glass

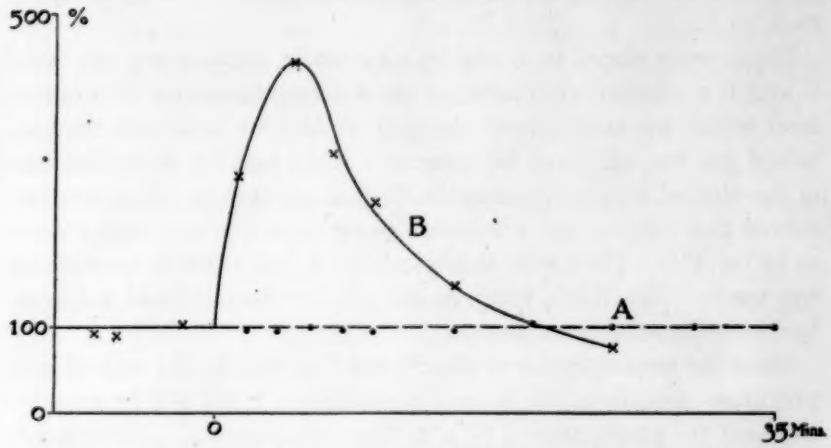



FIG. 1. Curves showing the respiration of *Salvia* petals first in tap water (horizontal line) and then in 7.3 per cent ether. Time is reckoned from the beginning of exposure to ether. The respiration of a control in tap water is shown by the broken line. The normal rate (which is taken as 100 per cent) corresponds to a change in pH value from 8.0 to 7.7 in 193 seconds. Temperature, 20° ± 1°C. Probable error less than 1.7 per cent of the mean. Curve A, average of six experiments; Curve B, average of four experiments.

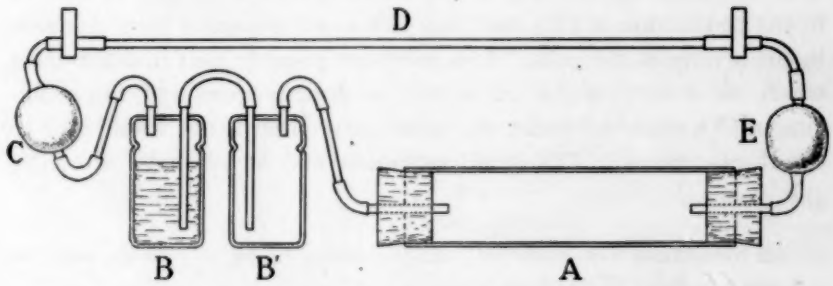



FIG. 2. Apparatus for the determination of oxygen consumption. Petals were placed in A; by alternate compression of the bulbs C and E the air in the apparatus was made to circulate. At the close of each experiment the tube D was removed and its contents were analyzed for oxygen.

and rubber)[7] to a bottle B, and a safety bottle B′. B contained strong $H_2SO_4$ and was connected to the rubber syringe bulb C. A was connected at the other end to the rubber syringe bulb E and to the long narrow glass tube D. When the bulbs C and E[8] were compressed, the air passed through A, B′, and B into C, and thence through D and E back to A.

Petals were placed in A and by alternately compressing the bulbs C and E a constant circulation of air was maintained for 10 minutes, after which the tube D was clamped off at both ends and the contained gas was analyzed for oxygen.[9] After making determinations of the normal oxygen consumption in this manner the petals were removed and exposed for 3 minutes to air saturated with ether vapor at 18° ± 1°C. They were then placed in A and a new determination was made. The $H_2SO_4$ removed the ether so that it could not interfere with the subsequent analysis.

Since the concentration of ether[10] was less than in the experiments previously described, the oxygen consumption could not be expected to equal the production of $CO_2$ in these experiments. Nevertheless the average of four experiments showed that ether increased the consumption of oxygen to 2.3 times the normal. The $CO_2$ production during the same time (in a saturated solution of ether), as measured by the methods previously discussed, amounted to three times the normal.

It is therefore evident that in this instance a high concentration of ether causes an increase in the consumption of oxygen as well as in the production of $CO_2$ and that this is accompanied by a decrease in the acidity of the cells. It is therefore possible that in other cases where the acidity of the cell cannot be directly measured, the evolution of $CO_2$ observed under the influence of ether is not wholly due to the displacement of $CO_2$ from carbonates and bicarbonates stored in the tissues.

[7] All connections were made with as little rubber tubing as possible, and care was taken to make all joints air-tight.

[8] These are provided with valves.

[9] The method employed was that described by Osterhout, W. J. V., *Am. J. Bot.*, 1918, v, 105.

[10] The concentration of ether constantly diminished during the 10 minutes.

SUMMARY.

In petals of *Salvia* high concentrations of ether cause an increase in oxygen consumption and in the production of $CO_2$, while at the same time a decrease occurs in the acidity of the cell contents.

# DECREASE OF PERMEABILITY AND ANTAGONISTIC EFFECTS CAUSED BY BILE SALTS.

By W. J. V. OSTERHOUT.

*(From the Laboratory of Plant Physiology, Harvard University, Cambridge.)*

(Received for publication, December 31, 1918.)

Agents which increase permeability have long been known but the discovery of substances which have the opposite effect is comparatively recent.[1] The number of such substances known at present (especially organic substances) is very small and it is therefore of interest to find that bile salts possess this property.

The discovery that some substances decrease permeability while others increase it led the writer to the idea that substances of one class may antagonize those of the other.[2] It was of interest to test this idea by ascertaining whether sodium taurocholate can antagonize NaCl (which produces only an increase of permeability). Some experiments on this subject are described in the present paper.

The experiments were made by determining the electrical conductivity of *Laminaria*[3] in solutions to which sodium taurocholate was added.

In the first experiments the bile salt was dissolved in sea water. The amounts added to 1,000 cc. of sea water varied from 0.8 to 1.5 gm. If the sodium taurocholate were pure, 1 gm. in 1,000 cc. would make the concentration about 0.002 M, but as its purity is doubtful the concentration cannot be accurately determined.[4]

After dissolving the sodium taurocholate the sea water was restored to the normal conductivity and made approximately neutral to litmus.

[1] Cf. Osterhout, W. J. V., *Bot. Gaz.*, 1915, lix, 317, 364.

[2] Osterhout, W. J. V., *Science*, 1915, xli, 255.

[3] For the method see Osterhout, W. J. V., *J. Biol. Chem.*, 1918, xxxvi, 557.

[4] The salt used was the purest obtainable.

At all the concentrations employed there was an immediate increase in resistance[5] followed by a fall, as illustrated in Fig. 1. Under the conditions of the experiment (temperature 19° ± 2°C.) the rise lasted about an hour. The effect is comparable with that of anesthetics[6] (ether, chloroform, and alcohol) as described by the writer. An in-

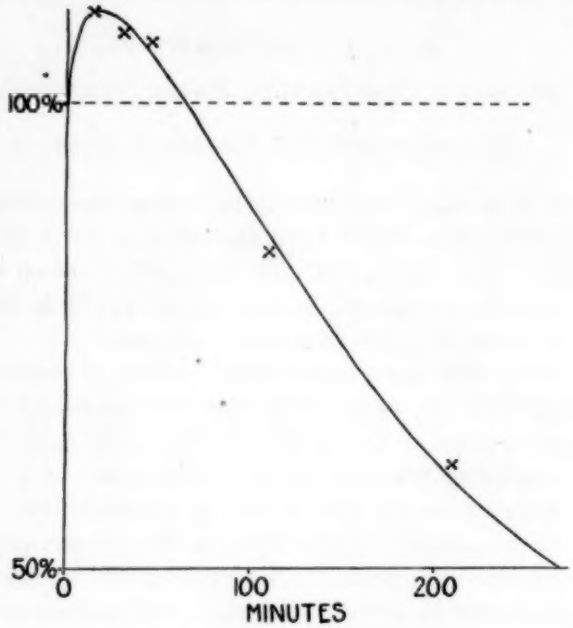



FIG. 1. Curve showing decrease of permeability (rise in net electrical resistance expressed as per cent of the control in sea water) of *Laminaria*, produced by adding 1 gm. of sodium taurocholate to 1,000 cc. of sea water (solid line). Control in sea water, dotted line. The resistance is expressed as per cent of the normal resistance in sea water, which is taken as 100 per cent. Average of two experiments; probable error less than 2.3 per cent.

crease in resistance was also observed with *Ulva rigida* and with *Rhodymenia palmata*.

In the experiments on antagonism the tissue was placed in a solution of NaCl 0.52 M to which various amounts of sodium taurocholate

[5] For convenience all the resistances are expressed as per cent of the normal resistance in sea water which is taken as 100 per cent.

[6] Cf. Osterhout, W. J. V., *Bot. Gaz.*, 1916, lxi, 148.

were added (all the solutions having the same conductivity as the sea water and being approximately neutral to litmus). The temperature was 18.5° ± 2.5°C.

The results are shown in Fig. 2. There is a gradual fall of resistance in all the solutions which continues until the death point (10 per

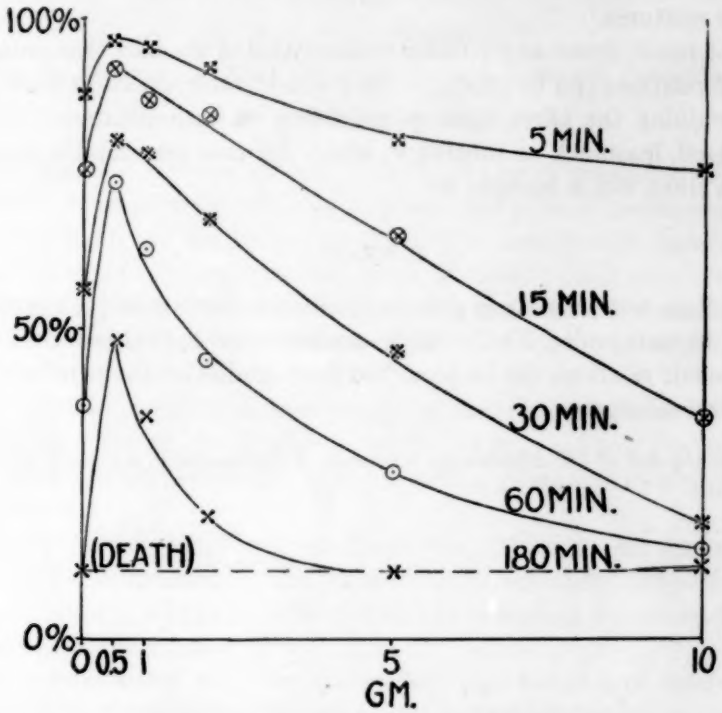



FIG. 2. Curves showing antagonism between NaCl and sodium taurocholate. The ordinates represent the net electrical resistance of *Laminaria* (expressed as per cent of the control in sea water which is taken as 100 per cent). The abscissæ represent the amount of sodium taurocholate added to 1,000 cc. of NaCl 0.52 M. Average of two experiments; probable error less than 5 per cent.

cent) is reached. In the solution containing 1,000 cc. of NaCl 0.52 M + 0.5 gm. of sodium taurocholate the fall of resistance is much slower, indicating that this is the most favorable mixture.

It should be emphasized that the effect is not an intermediate but an antagonistic one. By this is meant that the resistance is not

merely the algebraic mean between a rise in resistance produced by the bile salt and a fall produced by NaCl. A consideration of the lowest curve shows that at 180 minutes the tissue is dead in NaCl 0.52 M as well as in 1,000 cc. of NaCl 0.52 M + 10 gm. of sodium taurocholate, but in the mixture containing only 0.5 gm. of taurocholate it is not yet half dead, its resistance being much higher than in the other mixtures.[7]

The result serves as a striking confirmation of the idea that antagonistic relations can be predicted, to a considerable extent at least, by ascertaining the effect upon permeability of each substance taken by itself, inasmuch as substances which decrease permeability antagonize those which increase it.

## SUMMARY.

Sodium taurocholate is able to produce a decrease in permeability and to antagonize NaCl. This confirms the hypothesis that antagonistic relations can be predicted from studies on the permeability of pure substances.

[7] At the end of 180 minutes the resistance of the control in sea water was 100 per cent.

# A COMPARISON OF PERMEABILITY IN PLANT AND ANIMAL CELLS.

By W. J. V. OSTERHOUT.

*(From the Laboratory of Plant Physiology, Harvard University, Cambridge.)*

(Received for publication, January 9, 1919.)

In a study of the fundamental characteristics of protoplasm it is desirable to compare the behavior of plants and animals under conditions which are as similar as possible. The experiments here described were undertaken from this point of view. Their object was to discover whether the behavior of animals and plants is essentially similar with regard to certain aspects of permeability, injury, recovery, and death.

The method consisted in measuring the electrical conductivity of the tissues. Under the conditions of the experiment the electrical conductivity of the tissue may be regarded as a measure of its permeability to ions.

The plant chiefly employed was *Laminaria*, although other material was used for comparison.[1] The tissue was cut into disks and packed together to form a cylinder whose electrical resistance was measured as previously described.[2]

The animal tissue used for comparison was the skin of the frog (*Rana pipiens*), taken from the animal immediately after killing[3] and placed at once in the apparatus for measuring electrical resistance. Inasmuch as the skin has not sufficient mechanical rigidity to permit the same kind of manipulation which is possible in *Laminaria*, it was necessary to fasten each piece between two thin hard rubber disks (or between a rubber disk and one of thin celluloid), the disks being tied together by means of projections at the edges and the space between

[1] Osterhout, W. J. V., *J. Gen. Physiol.*, 1918–19, i, 299.

[2] Osterhout, W. J. V., *J. Biol. Chem.*, 1918, xxxvi, 557.

[3] The animals were killed by decapitation. The use of anesthetics must be avoided as they may injure the skin.

them being partially filled with vaseline.[4] The disks thus prepared were used in the same way as the disks of *Laminaria*. The conditions were therefore strictly comparable. In order to make this doubly sure, pieces of *Laminaria* of the same size as the pieces of frog skin were fastened between disks of hard rubber in the same manner and used for comparison. The resistance of a pair of disks containing frog skin was in the neighborhood of three times that of a pair of disks containing *Laminaria*. Usually five pairs of disks with frog skin were placed together to form a cylinder whose resistance was measured.[5]

The measurement of the resistance of the frog skin was less accurate than that of *Laminaria*. The point of minimum sound in the telephone was more indefinite (especially with live tissue). The resistance of the control was not constant as in the case of *Laminaria*. The controls of frog skin were placed in sea water + four volumes of distilled water, which was taken as approximately isotonic (for convenience this will be called 0.2 sea water). In this solution the resistance sometimes remained constant for some time, but more often it rose somewhat and finally became constant for a time or else began slowly to fall.[6] Increasing or diminishing the proportion of distilled water did not help. Soaking the frog in the solution for an hour before removing the skin made no decided difference. In spite of these difficulties it was possible to select from several lots of material some whose resistance did not change much in 0.2 sea water, and thus to obtain consistent results. It was found desirable to express all the net resistances as per cent of the controls.[7]

[4] Cf. Osterhout, W. J. V., *J. Biol. Chem.*, 1918, xxxvi, 557. The method shown in Fig. 7 was usually employed. The apparatus described as type B was also used and gave similar results.

[5] Five pairs of disks containing *Laminaria* had about the same resistance as a cylinder of *Laminaria* tissue consisting of 80 pieces with only one rubber disk at each end.

[6] Regarding changes in the conductivity of frog skin, under various conditions, see Höber, R., Physikalische Chemie der Zelle und der Gewebe, Leipsic, 4th edition, 1915, 441.

[7] For example, a piece of frog skin was placed in NaCl and after a given time the resistance was measured; this was divided by the resistance of a control in 0.2 sea water measured at the same time (both lots having been placed in the solution at the same moment). In 0.2 sea water the skin remained alive for several hours.

One of the most striking results observed in studying the electrical resistance of *Laminaria* is the fact that some substances (as $CaCl_2$) decrease permeability while others (as NaCl) increase it. It is of interest to see whether animal tissue behaves in the same manner.

When *Laminaria* is placed in a solution of $CaCl_2$ (of the same conductivity as the sea water) the resistance[8] rapidly rises, often as much as 65 per cent; in $LaNO_3$ it rises even higher (90 per cent or more),[9] while in $MgCl_2$ the rise is less (up to 10 per cent). The rise is succeeded by a fall, which continues until death. The resistance has then dropped to about 10 per cent of the normal resistance.[10]

When frog skin is placed in $CaCl_2$ of the same conductivity as 0.2 sea water (about 0.056 M $CaCl_2$), the resistance rises rapidly, just as in the case of *Laminaria*, though not as high (up to 40 per cent). It then begins to fall, and continues to do so until the death point is reached[11] at about 10 per cent of the normal resistance. In $LaNO_3$ the rise is greater (up to 90 per cent), and in $MgCl_2$ it is less (up to 10 per cent). It will be seen that the behavior of frog skin toward these agents closely resembles that of *Laminaria*.

Acid (HCl) causes a rapid rise of resistance in *Laminaria*,[12] followed by a rapid fall. This is also the case with frog skin placed in a solution of HCl of the same conductivity as 0.2 sea water (about 0.024 M HCl) or in this solution diluted with 0.2 sea water. The use of HCl is attended by some complications which will be discussed in a later paper.

There is another group of substances, such as NaCl and KCl, which produce no rise in the resistance of *Laminaria*. On transferring tissue from sea water to solutions of these substances (of the same conductivity as sea water) there is a fall of resistance which continues until the death point is reached. The same is true of frog skin (using 0.2 sea water) except that the fall of resistance is much slower than in the case of *Laminaria*. It is, however, completely paralleled by the slow fall of resistance found in some other plant tissues, such as those of the dulse (*Rhodymenia palmata*).[1]

[8] Osterhout, W. J. V., *Bot. Gaz.*, 1915, lix, 317.

[9] Osterhout, W. J. V., *Bot. Gaz.*, 1915, lix, 464.

[10] The resistance of the apparatus is subtracted in all cases.

[11] The death point is not so well defined as with *Laminaria*.

[12] Cf. Osterhout, W. J. V., *J. Biol. Chem.*, 1914, xix, 493.

In mixtures of NaCl and $CaCl_2$ in suitable proportions[13] *Laminaria* remains alive longer than in pure NaCl or in pure $CaCl_2$. This is also true of frog skin, although the result is not as marked as in the case of *Laminaria;* this is perhaps due to the fact that NaCl is less toxic to frog skin.

Of special interest is the behavior of anesthetics. In the case of *Laminaria* they produce two effects, a reversible (anesthetic) action consisting in a decrease of permeability and an irreversible (toxic) action consisting in an increase of permeability.[14] On placing tissue in sea water containing suitable amounts of ether (1 per cent), chloroform (0.1 per cent), or chloral hydrate (0.1 per cent), the resistance rises and this condition is maintained for some time. With increasing concentration a point is soon reached at which the resistance rises rapidly to a maximum and then falls rather rapidly. When it has fallen below the normal there is little or no recovery on replacing it in sea water. With alcohol such recovery is possible.

The same is true of frog skin (using 0.2 sea water) but the effect is even more striking, the rise of resistance being greater and occurring at lower concentrations. In respect to recovery we find the same difference between ether, chloroform, and chloral hydrate on the one hand and alcohol on the other.

The method of measuring electrical resistance enables us to study the dynamics of the death process. It has been shown that when *Laminaria* dies in a solution of NaCl the process follows more or less closely the curve of a monomolecular reaction.[15] The same is true of frog skin. In both cases we are led to the assumption that the process of death is one which is always going on during the normal life of the cell and that it is accelerated by the toxic agent. It is also found, in both *Laminaria* and frog skin, that if the death process has not proceeded too far a complete or partial recovery is possible when the tissues are removed from the toxic solution and returned to sea water (or 0.2 sea water).

In both cases it appears that permeability is a delicate and accurate index of the vitality[16] of the protoplasm and that agents which pro-

[13] Osterhout, W. J. V., *Proc. Am. Phil. Soc.*, 1916, lv, 533.
[14] Osterhout, W. J. V., *Bot. Gaz.*, 1916, lxi, 148.
[15] Osterhout, W. J. V., *J. Biol. Chem.*, 1917, xxxi, 585.
[16] Osterhout, W. J. V., *Science*, 1914, xl, 488.

duce injury increase permeability. The amount of increase may be regarded as a measure of the amount of injury. A quantitative basis is thereby furnished for such conceptions as death, injury, recovery, and vitality.

The striking agreement between the behavior of frog skin and that of *Laminaria*, as well as of other plants previously studied,[1] strongly confirms the idea that the ideas which have been developed from the study of *Laminaria* are of general application. These ideas have been tested by the use of diverse methods.[17]

The general outcome of these studies reveals a marked amount of agreement, and it would seem that the physiological characteristics which they bring to light belong to the fundamental properties of protoplasm.

## SUMMARY.

Quantitative studies show a striking agreement between frog skin and plant tissues in respect to certain important aspects of permeability, antagonism, injury, recovery, and death.

[17] Osterhout, W. J. V., *Science*, 1917, xlv, 97; *J. Biol. Chem.*, 1918, xxxvi, 485.

# THE RELATION BETWEEN THE OXYGEN CONCENTRATION AND RATE OF REDUCTION OF METHYLENE BLUE BY MILK.

By E. NEWTON HARVEY.

(*From the Physiological Laboratory, Princeton University, Princeton.*)

(Received for publication, December 27, 1918.)

After the discovery of the reducing power of milk[1] and the proof that its reducing action on methylene blue (decolorization) is not due to bacteria but to a reducing enzyme,[2] Bach,[3] in a series of researches, undertook the study of reducing enzymes in various tissues. Schardinger[1] had originally found that fresh cow's milk will reduce methylene blue in the presence of an aldehyde but not in its absence and not if the milk had previously been boiled. In one paper Bach[4] showed that nitrates could also be reduced to nitrites by Schardinger's enzyme of milk and he studied the effect (on reduction) of nitrate, aldehyde, and enzyme concentration, as well as the influence of temperature. As the nitrate is still further reduced, the quantitative results obtained by determining the amount of nitrate formed at successive intervals of time after mixing varying quantities of nitrate, or aldehyde, or milk, are not of much value. They do show, however, that rate of nitrite formation increases with, but is not proportional to nitrate concentration or aldehyde concentration. Bach found also that nitrite formation is proportional to enzyme concentration, at least in the early stages of the reduction. He did not study the effect of oxygen concentration in milk on the reduction of nitrate.

[1] Schardinger, F., *Z. Untersuch. Nahrungs-u. Genussmittel*, 1902, v, 1113; *Chem. Ztng.*, 1904, xxviii, 704.

[2] Trommsdorff, R., *Centralbl. Bacteriol., 1te Abt., Orig.*, 1909, xlix, 291.

[3] Bach, A., *Biochem. Z.*, 1911, xxxi, 443; 1911, xxxiii, 282; 1912, xxxviii, 154; 1913, lii, 412.

[4] Bach, A., *Biochem. Z.*, 1911, xxxiii, 282.

This paper deals with the reduction of methylene blue by milk and acetaldehyde under varying partial pressures of oxygen. The study was undertaken through the observation that milk will reduce oxyluciferin to luciferin, the light-producing substance of luminous animals, or methylene blue to leucomethylene blue, only if the milk and reducible material stand for some time.[5] The reduction does not occur in either case if the mixtures are continually shaken with air. It appears that time is necessary for using up the dissolved oxygen present. If the oxygen is removed from a milk-acetaldehyde-methylene blue mixture by exhaustion with an air pump, decolorization (reduction) occurs almost immediately. Without exhaustion of the air such a mixture must remain undisturbed for over an hour at room temperature before reduction occurs.

Preliminary experiments showed that the amount of reducing enzyme varied in different samples of milk. The addition of 1 cc. M acetaldehyde to 10 cc. of one sample of milk gave the quickest reducing action. Either more or less aldehyde increased the time. Methylene blue was added till a blue color in the milk was marked (0.1 cc. 0.01 M ( = 0.319 per cent) methylene blue was used). With less methylene blue slightly less time is required. The standard mixture was therefore 10 cc. of milk and 1 cc. M acetaldehyde + 0.1 cc. 0.01 M methylene blue. If shaken with air (21 per cent oxygen) and set aside in a test-tube at 20°C., the blue color in one particular case completely disappeared, except at the surface in contact with air, in 43 minutes. If shaken with air containing only half the normal amount of oxygen, decolorization occurs in 23 minutes, about one-half the time. The times for decolorization have been determined for other concentrations of oxygen and are given in Fig. 1 where times for decolorization are plotted along ordinates and oxygen concentrations along the abscissæ.

The reduction in concentration of oxygen was accomplished by partially exhausting the air over the milk-acetaldehyde-methylene blue mixture in a large test-tube with an air pump, at the same time shaking the mixture vigorously. Tubes which have been brought into equilibrium with air in partial vacua can be removed to air under

[5] Harvey, E. N., *J. Gen. Physiol.*, 1918–19, i, 133.

atmospheric pressure and placed in a thermostat at 20° with safety because the diffusion of dissolved oxygen from the surface into the solution is very slow, if the tubes are undisturbed.

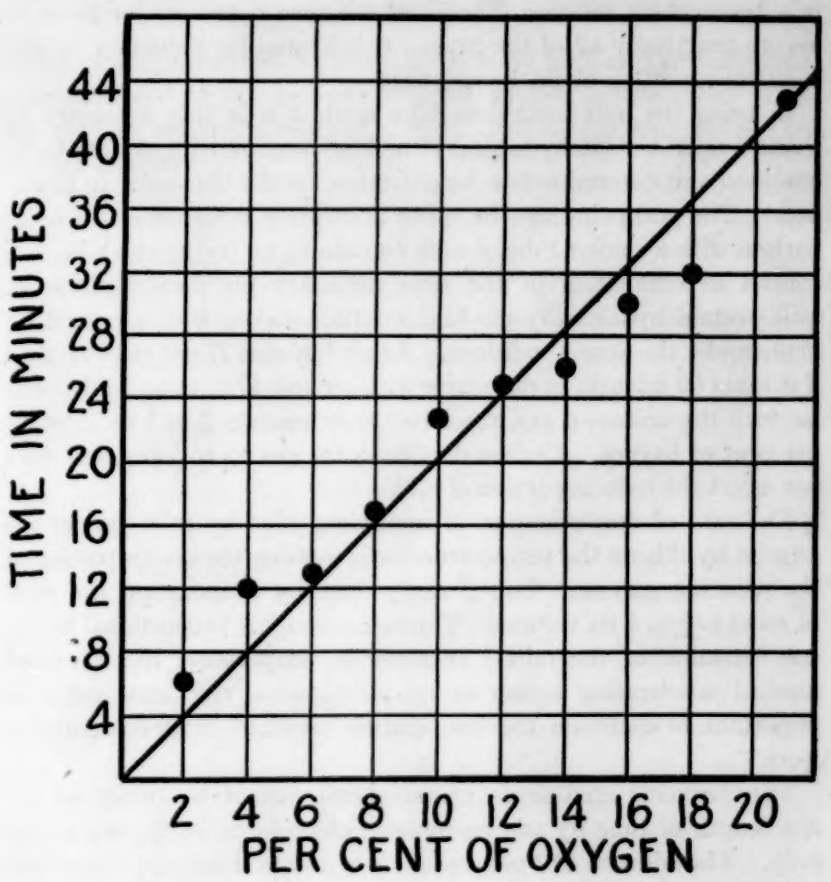



FIG. 1. Time necessary for reduction of methylene blue in varying concentrations of oxygen.

It will be noted from Fig. 1[6] that the curve is practically a straight line and the time for decolorization proportional to the oxygen concentration. It is obvious that this method can be used for determin-

[6] The results of this chart were obtained by Mr. J. P. Kelly, Princeton University, who undertook the determination at my suggestion.

ing the oxygen content in gaseous mixtures by use of the indicator methylene blue. The procedure differs somewhat from the indicator method recently described by Osterhout,[7] where organisms are placed in a hemocyanin solution (blue) and the time necessary for them to use up practically all of the oxygen is indicated by reduction to the colorless condition of the hemocyanin.

In using the milk-methylene blue method it is only necessary to shake a milk-acetaldehyde-methylene blue mixture with the gas to be analyzed and determine how long it takes for the blue color to disappear. The end-point can be quite accurately determined by comparison with a similar tube of milk containing no methylene blue. A control determination of the time necessary for decolorization of milk-acetaldehyde-methylene blue mixture shaken with air must be made under the same conditions. As air contains 21 per cent oxygen, if it takes 60 minutes to decolorize with air and 40 minutes to decolorize with the unknown gas, the latter must contain $\frac{40}{60}$ or $\frac{2}{3}$ of 21 = 14 per cent of oxygen. Carbon dioxide in the gas up to 5 per cent does not affect the reducing action of milk.

The rate of decolorization of methylene blue by milk can be increased by raising the temperature or increasing the concentration of the reducing enzyme. This is easily done by evaporating the milk *in vacuo* to $\frac{1}{3}$ to $\frac{1}{4}$ its volume. The rate is roughly proportional to the concentration of the milk. Increase in temperature has the same marked accelerating action as on all chemical reactions, and it is important to maintain the temperature constant in all comparative work.

The reducing enzyme is unstable and cannot be preserved for any length of time by adding toluene, chloroform, or thymol to the milk. The addition of 2 per cent NaF to milk will prevent the growth of bacteria without affecting its reducing powers during a period of 2 months. Some samples of canned evaporated milk which I examined did not exhibit a reducing action. Since colloidal platinum and formic acid reduce methylene blue rapidly, imitating the milk-aldehyde reducing action,[8] it is likely that a protected platinum solution

[7] Osterhout, W. J. V., *J. Gen. Physiol.*, 1918–19, i, 167.

[8] Bredig, G., and Sommer, F., *Z. physik. Chem.*, 1910, lxx, 34.

might take the place of milk as a more stable medium for oxygen determinations.

## SUMMARY.

The rate of reduction of methylene blue by milk and acetaldehyde is proportional to the concentration of oxygen in the milk. This fact may be made the basis of a method of determining oxygen in gaseous mixtures.

# INFLUENCE OF TEMPERATURE AND HYDROGEN ION CONCENTRATION UPON THE SPORE CYCLE OF BACILLUS SUBTILIS.

By ARAO ITANO and JAMES NEILL

*(From the Department of Microbiology, Experiment Station, Massachusetts Agricultural College, Amherst.)*

(Received for publication, December 31, 1918.)

The investigation here reported deals with the influence of hydrogen ion concentration at different temperatures upon the spore cycle of *Bacillus subtilis*.[1] This microorganism was chosen because it is a classical spore-bearing species, which had been used in our previous investigations on the relation of the hydrogen ion concentration of the medium to proteolytic activity.

TABLE I.

*Limiting Reactions.**

| Organism. | pH | Author. |
|---|---|---|
| *B. coli* | 5.0 | Michaelis and Marcora.† |
| " " (low gas ratio group) | 4.3–5.3 | Clark, 1915.‡ |
| Streptococcus, Group I | 4.6–4.8 | Ayers, 1916.§ |
| " " II | 5.5–6.0 | " 1916.§ |
| *S. erysipelatis* | 4.8 | Itano, 1916.‖ |
| *B. subtilis* (acid limit) | 4.2 | " 1916.‖ |
| " " (alkali limit) | 9.4 | " 1916.‖ |

* Clark, W. M., and Lubs, H. A., *J. Bacteriol.*, 1917, ii, 1, 222.

† Michaelis, L., and Marcora, F., *Immunitätsforsch., Orig.*, 1912, xiv, 170.

‡ Clark, W. M., *J. Biol. Chem.*, 1915, xxii, 87.

§ Ayers, S. H., *J. Bacteriol.*, 1916, i, 84.

‖ Itano, A., *Massachusetts Agric. Exp. Sta., Bull. 167*, 1916, 139.

[1] The same strain of this species was used as in the previous investigations on proteolytic activity; the strain was obtained from the American Museum of Natural History.

We have found no references in the literature to the particular problem involved, but it may be interesting to present in tabular form the limiting reactions of the medium for some bacteria.

Table I indicates that these organisms survive in a certain limited range of hydrogen ion concentrations, and that slight changes in the concentration of the hydrogen ion influence the organism to a great extent.

## *Method of Procedure.*

*Preparation of Media.*—The media used in this investigation had the same composition and were prepared in the same way as those described in a previous article.[2] The pH was determined electrometrically and was found to be as shown in Table II.

TABLE II.

*Preparation of Media of Different pH Values.*

| pH desired..... | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| pH found..... | 1.2 | 2.0 | 3.0 | 4.1 | 5.2 | 6.1 | 7.1 | 8.2 | 9.2 | 10.1 | 11.0 | 11.5 | 12.7 |

Throughout the following account, we have used the approximate figures in referring to the various pH values; *i.e.*, desired pH is used to denote the hydrogen ion concentration of the broth.

*Method Used in Obtaining Free Spores.*—The spores of the organism were obtained as follows: A portion of a young agar culture of *Bacillus subtilis* was emulsified in sterile 0.85 per cent salt solution. The emulsion was then transferred to a Roux flask containing standard agar, and the organisms were distributed over the surface of the medium. The flask was incubated at 30°C. for 15 days and was then kept at 25°C. for 3 weeks. At the time of the experiment the culture contained practically nothing but free spores.

*Preparation of Moist Chambers.*—Two moist chambers were made for each pH value for each temperature. The chambers were prepared in the usual way, using paraffin of high melting point to seal the ring to the slide. By means of a platinum loop 2 mm. in diameter,

[2] Itano, A., *Massachusetts Agric. Exp. Sta., Bull. 167*, 1916, 164.

a drop of medium of each pH value was put upon a sterile cover-slip which was then carefully sealed upon the ring of the moist chamber. The moist chamber preparations were then kept for 12 hours at the temperature to be used in the subsequent incubation.

*Preparation of Dilute Emulsion of Spores.*—Two drops of the medium of each pH value were put in the cells of sterile concave slides in sterile Petri dishes. With a straight needle a small inoculum of spores was put into the first drop of broth of each pH value. From this drop the second drop of the same pH value was then inoculated by use of the straight needle. The second drop was used as the source of the final inoculum.

Procedure in this manner with the medium of each pH value gave a very dilute emulsion of free spores in broth of each pH value. By this procedure we obtained for the inoculation of the hanging drops a satisfactory source for a small number of spores, well washed of all metabolic products; moreover, any of the medium carried over with the final inoculum was of the same pH value and of the same composition as the drop inoculated.

*Inoculation of Drops.*—The hanging drops of the moist chamber preparations were then inoculated; those of each pH value were inoculated from the dilute emulsion of spores in broth of the same pH value. By using a straight needle for transferring and by stirring the inoculated drop, a drop was obtained containing a satisfactory number of spores well distributed throughout the drop.

*Examination of Preparations and Conditions of Observation.*—Immediately after inoculation the preparations were placed under a microscope and examined for absence of vegetative forms and for even distribution of the spores. After examination the preparations were placed at the temperature of incubation. Observations were made at intervals of 30 minutes for the first 5 hours and then at intervals of 1 hour.

## RESULTS.

*Series I (5°C.).*—This series (pH 1 to 13) was incubated at 5°C. for 20 days.[3] No apparent change took place except a slight swelling of the spores in all hydrogen ion concentrations. This temperature

[3] The cold storage room of the Dairy Department of this institution was used.

seems to be near the limiting temperature for spore germination of *Bacillus subtilis*. Schreiber[4] reports the germination of spores of this organism at 8°C. after 7 days, but states that the spore cycle is not completed at this temperature.

*Series II (25°C.).*—This series was kept at room temperature, which varied during the time of the experiment from 23.5–25°C. The observations and results are given in Table III.

TABLE III.

*Time in Hours Required by B. subtilis to Reach Various Stages of Development at Different pH Values. (Temperature Approximately 25°C.)*

| pH | Spore. | | Vegetative cell. | | | | Spore. | |
|---|---|---|---|---|---|---|---|---|
| | Swollen. | Germination begins. | Single. | Chains. | Granule. | Refractive body. | Endospore. | Free spore. |
| | *hrs.* | *hrs.* | *hrs.* | *hrs.* | *hrs.* | *hrs.* | *hrs.* | *hrs.* |
| 1 | S | | | | | | | |
| 2 | " | | | | | | | |
| 3 | " | | | | | | | |
| 4 | " | | | | | | | |
| 5 | 2.5 | 7.5 | 10.0 | 12.0 | 25.0 | 30.0 | 32.0 | 50.0 |
| 6 | 2.5 | 6.0 | 8.0 | 9.0 | 22.0 | 30.0 | 31.0 | 46.0 |
| 7 | 2.0 | 4.0 | 5.0 | 6.0 | 20.0 | 26.0 | 29.0 | 39.0 |
| 8 | 2.0 | 3.0 | 4.0 | 5.0 | 16.0 | 41.0 | 51.0 | 65.0 |
| 9 | 3.0 | 7.0 | 10.0 | 13.0 | 20.0 | 44.0 | 52.0 | 66.0 |
| 10 | 3.5 | 8.0 | 11.0 | 15.0 | 22.0 | 25.0 | 31.0 | 39.0* |
| 11 | S | | | | | | | |
| 12 | " | | | | | | | |
| 13 | " | | | | | | | |

Figures represent average of four experiments.

S indicates no apparent change except slight swelling and a certain loss of refrangibility.

* Few spores germinated; chains are short; not all cells formed spores.

The results in Table III show that at 25°C. the spores of *Bacillus subtilis* germinated in broth at pH values from 5 to 10, while higher and lower concentrations of hydrogen ions inhibited their development. At pH 7 and pH 8, which are nearest the optimum hydrogen ion concentration for this organism, germination took place in the shortest time and multiplication was most rapid.

[4] Schreiber, O., *Centralbl. Bakteriol., Ite Abt.*, 1896, xx, 432.

An interesting phenomenon was observed in the broth at pH 5, 6, and 9. At the time given in Table III only a few spores had germinated. These first vegetative cells did not exhibit the characteristic motility of this species, neither did rapid multiplication begin at once. Several hours later, however, after most of the spores had germinated the vegetative cells became actively motile and multiplication became much more rapid, indicating that by the life processes of the organisms

TABLE IV.

*Time in Hours Required by B. subtilis to Reach Various Stages of Development at Different pH Values. (Temperature 37°C.)*

| pH | Spore. | | Vegetative cell. | | | | Spore. | |
|---|---|---|---|---|---|---|---|---|
| | Swollen. | Germination begins. | Single. | Chains. | Granule. | Refractive body. | Endo-spore. | Free spore. |
| | *hrs.* | *hrs.* | *hrs.* | *hrs.* | *hrs.* | *hrs.* | *hrs.* | *hrs.* |
| 1 | S | | | | | | | |
| 2 | " | | | | | | | |
| 3 | " | | | | | | | |
| 4 | " | | | | | | | |
| 5 | 2.0 | 11.0 | 13.0 | 15.0 | 20.0 | 24.0 | 27.0 | 32.0 |
| 6 | 1.5 | 8.0 | 9.0 | 10.0 | 14.0 | 19.0 | 22.0 | 28.0 |
| 7 | 1.5 | 5.0 | 7.0 | 9.0 | 12.0 | 17.0 | 19.0 | 26.0 |
| 8 | 1.5 | 3.5 | 6.0 | 8.0 | 10.0 | 12.0 | 13.0 | 20.0 |
| 9 | 1.5 | 4.0 | 5.0 | 7.0 | 8.0 | 10.0 | 14.0 | 23.0 |
| 10 | 1.5 | 11.0 | 12.0 | 14.0 | 15.0 | 18.0 | 25.0 | 35.0 |
| 11 | S | | | | | | | |
| 12 | " | | | | | | | |
| 13 | " | | | | | | | |

Figures represent average of four experiments.

S indicates no apparent change except slight swelling and a certain loss of refrangibility.

the reaction of the medium was approaching the optimum. In view of facts shown in a previous publication,[5] this behavior may be explained as a manifestation of the beginning of the automatic adjustment of the medium.

The behavior in pH 10 was also interesting. Only a few of the spores germinated and these passed into the spore stage in a comparatively short time without much further multiplication.

[5] Itano, A., *Massachusetts Agric. Exp. Sta., Bull. 167*, 1916, 174.

*Series III (37°C.).*—This series was kept in the incubator at 37°C. The results are given in Table IV.

In general Table IV shows the same results as Table III, except that the rate of completion of the cycle was accelerated at the higher

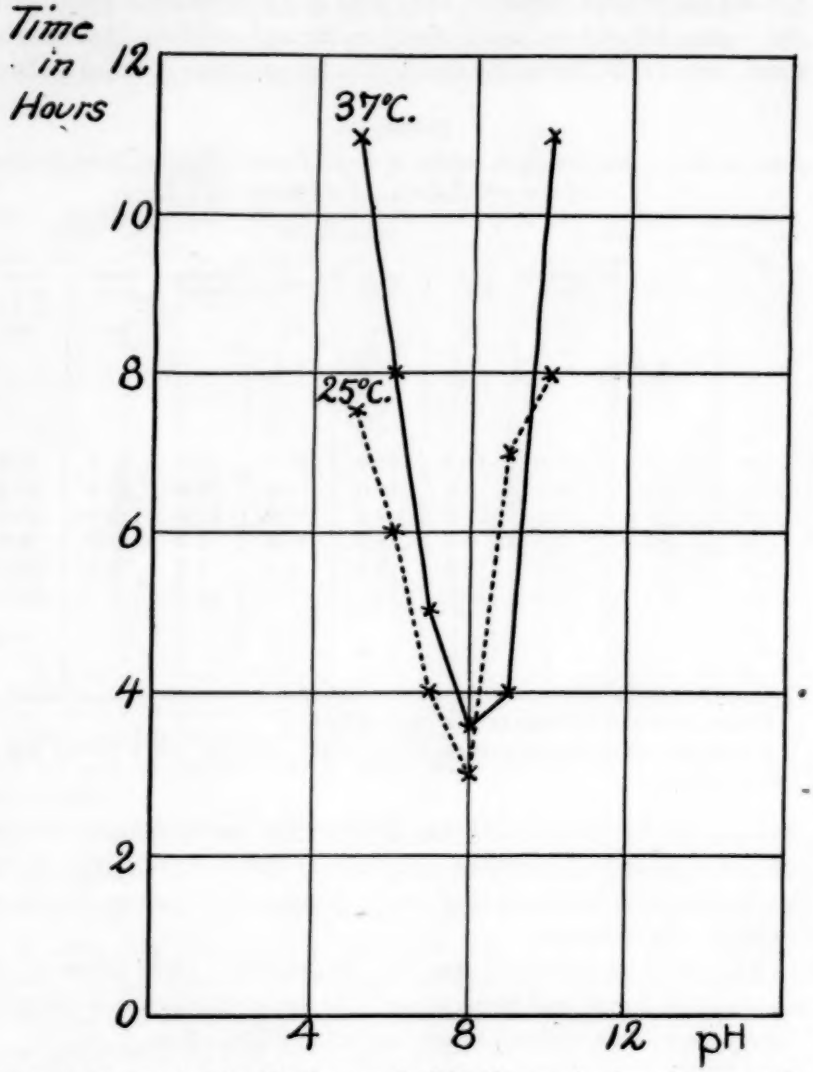



FIG. 1. Curves showing the time required for the germination of spores of *B. subtilis* in broth of different pH values at 25° and 37°C.

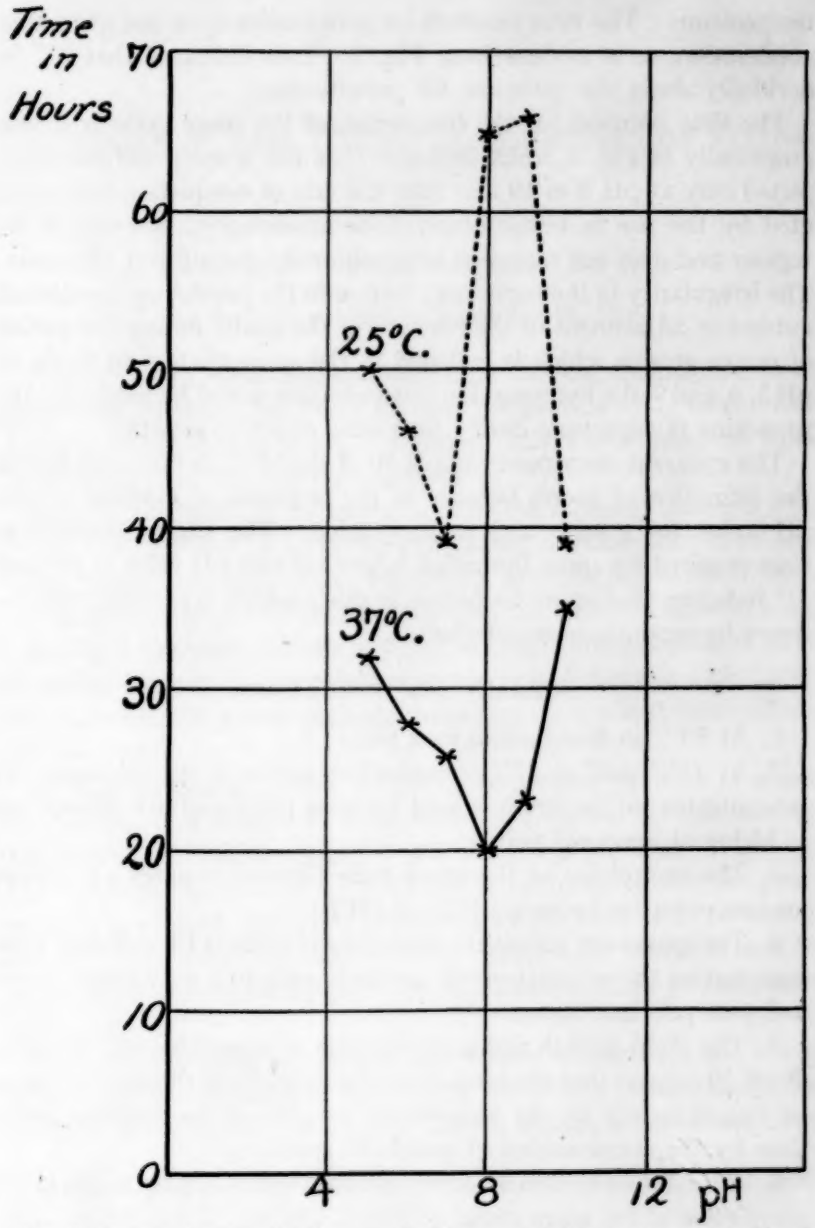



FIG. 2. Curves showing the time required for completion of the spore cycle of *B. subtilis* in broth of different pH values at 25° and 37°C.

temperature. The time required for germination does not show this acceleration, as is evident from Fig. 1. This indicates that 37° is decidedly above the optimum for germination.

The time required for the completion of the spore cycle is shown graphically in Fig. 2, which indicates that the spore cycle was completed only at pH 5 to 10 and that the rate of completion is accelerated by the rise in temperature. The acceleration, however, is irregular and does not maintain any uniformity throughout the series. The irregularity in the curve may be due to the previously mentioned automatic adjustment of the reaction of the media during the period of active growth which is included in the spore cycle. In broth of pH 5, 6, and 9, the hydrogen ion concentration would be gradually approaching the optimum during the period of active growth.

The apparent discrepancy in pH 10 of the 25°C. is probably due to the formation of spores because of the unfitness of medium of this pH value for growth and multiplication. The slight difference in time required for spore formation in broth of this pH value at 25° and 37° indicates that spore formation in this medium is probably not induced by products of metabolism.

## SUMMARY AND CONCLUSIONS.

1. At 5°C. no germination took place.

2. At 25°C. and at 37°C. germination occurs if the hydrogen ion concentration of the broth is kept between pH 5 and pH 10, but not at higher or lower pH values.

3. The completion of the spore cycle likewise requires a hydrogen ion concentration between pH 5 and pH 10.

4. The spores can germinate when the pH value is 10, although after germination the vegetative cells multiply only to a very slight extent and soon pass into spores.

5. The slight growth and multiplication of vegetative cells in broth of pH 10 suggest that the formation of endospores in this medium must be caused largely by the unfavorable reaction of the medium rather than by the accumulation of metabolic products.

6. Automatic adjustment of the medium seems to play a rôle in the completion of the spore cycle.

7. The results are not only of theoretical importance but they have a practical application to the preservation of food by canning and by other methods.

# BIOELEMENTS; THE CHEMICAL ELEMENTS OF LIVING MATTER.

By INGO W. D. HACKH.

*(From the College of Physicians and Surgeons, San Francisco.)*

(Received for publication, November 25, 1918.)

Among the 87 known elements less than one-half (namely 34) have been found to enter into living matter. Of these only 17 seem to be essential to life, and of these 17 not more than 4 elements form from 97 to 99 per cent of the living organism.

In order to illustrate the importance of the different elements Fig. 1 and Tables I and II have been prepared, showing that some elements occur invariably, others frequently, and still others are of such rare occurrence that their presence in living matter seems adventitious.

In Fig. 1 the new periodic system[1] has been utilized to show that all the bioelements occupy neighboring places and that the majority of them possess low atomic weights, belonging to the first two periods of the system.

An approximation to the ultimate composition of man is given in Column 2 of Table I, while Column 3 shows the elementary composition of long leaf pine, calculated from the data of Little.[2] The chemical character of the mammal in comparison with the gymnosperm is evident, the latter containing 99 per cent of C, H, and O and only very small amounts of other elements, of which Al and Si are the predominant ones (due to the abundance of these two elements in the soil).

With the exception of oxygen, no element enters into the living organism as a free element, but only in the form of compounds, the majority of these being non-polar; that is, organic compounds. For

[1] Hackh, I. W. D., *J. Am. Chem. Soc.*, 1918, xl, 1023; *Am. J. Sc.*, 1918, xlvi, 481.

[2] Little, A. D., *J. Ind. and Eng. Chem.*, 1916, viii, 102.

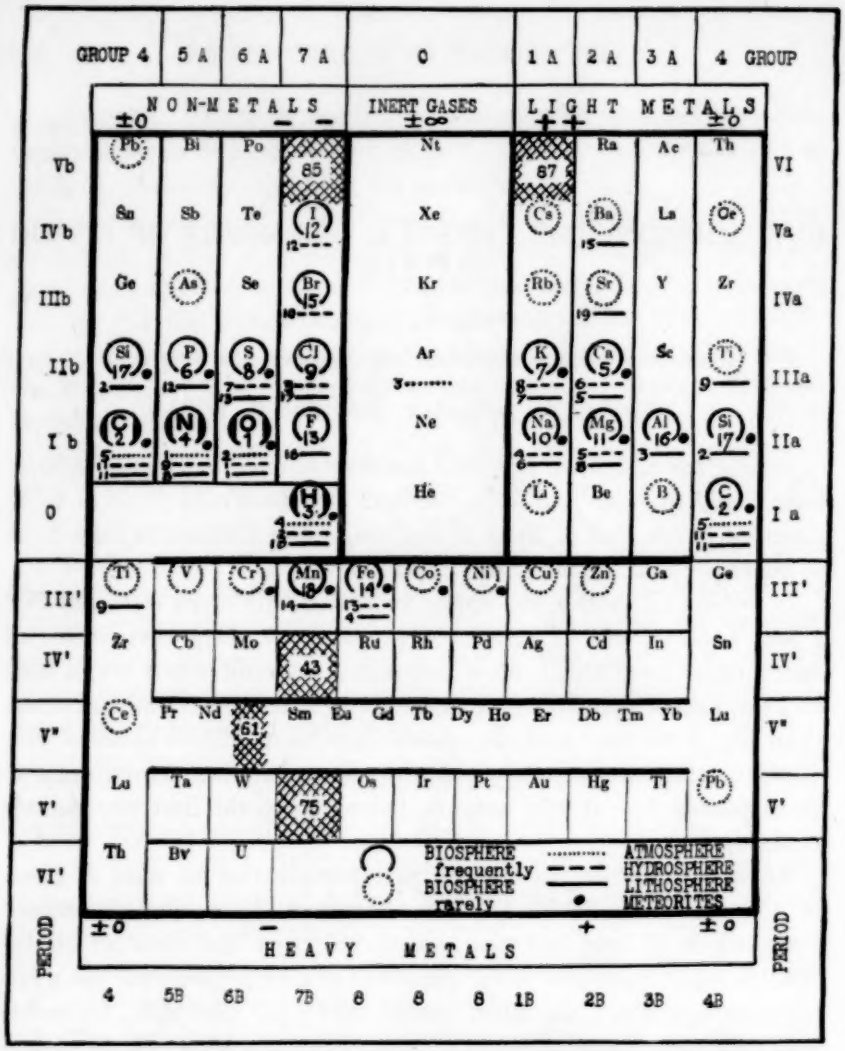



FIG. 1. Bioelements and the periodic system. Bioelements invariably or frequently present in living organisms are indicated by a continuous arc; those which occur variably and rarely by a broken circle. The numbers indicate the order of their relative percentage. The elements of the lithosphere, hydrosphere, and atmosphere are also indicated by a continuous, broken, or dotted line. The elements occurring in meteorites are shown by a dot. The numbers indicate their relative abundance. This chart of the periodic system divides the elements into non-metals, light metals, and heavy metals. Missing elements are those of atomic numbers 43, 61, 75, 85, and 87, while Po = polonium, Ac = actinium, and Bv = brevium are radioactive substances, of which so far no long lived isotope is known. (See Hackh, I. W. D., *Am. J. Sc.*, 1918, xlvi, 481; *J. Am. Chem. Soc.*, 1908, xl, 1023.)

comparison the average composition of these compounds or foods is given in Columns 4, 5, 6, and 7 of Table I.

Regarding the distribution of the elements in the biosphere, there are the nine essential elements which are invariably present in the cell: C, H, O, N, P, S, Mg, Fe, and K, while eight more seem also to be important, for they are always present in small amounts in plant

TABLE I.

*Distribution of Bioelements.*

| Order. | Mammals. | | Gymnosperms. | | Average composition of foods. | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Carbohydrates. | | Fats. | | Proteins. | | Fresh water. | |
| (1) | (2) *per cent* | | (3) *per cent* | | (4) *per cent* | | (5) *per cent* | | (6) *per cent* | | (7) *per cent* | |
| 1 | O | 62.43 | C | 53.96 | O | 49.38 | C | 69.05 | C | 51.3 | O | 88.80 |
| 2 | C | 21.15 | O | 38.65 | C | 44.44 | O | 17.90 | O | 22.4 | H | 11.16 |
| 3 | H | 9.86 | H | 7.13 | H | 6.18 | H | 10.00 | N | 17.8 | S | 0.018 |
| 4 | N | 3.10 | Al | 0.065 | | — | P | 2.13 | H | 6.9 | C | 0.005 |
| 5 | Ca | 1.90 | Si | 0.057 | | — | N | 0.61 | S | 0.8 | Ca | 0.004 |
| 6 | P | 0.95 | S | 0.052 | | — | S | 0.31 | P | 0.7 | Mg | 0.003 |
| 7 | K | 0.23 | Fe | 0.030 | | — | | — | Fe | 0.1 | N | 0.002 |
| 8 | S | 0.16 | N | 0.030 | | — | | — | | — | Fe | 0.002 |
| 9 | Cl | 0.08 | Ca | 0.007 | | — | | — | | — | P | 0.001 |
| 10 | Na | 0.080 | K | 0.006 | | — | | — | | — | Na | 0.001 |
| 11 | Mg | 0.027 | P | 0.005 | | — | | — | | — | K | 0.001 |
| 12 | I | 0.014 | Mg | 0.003 | | — | | — | | — | Remainder | 0.003 |
| 13 | F | 0.009 | Cl | 0.002 | | — | | — | | — | | — |
| 14 | Fe | 0.005 | Na | 0.001 | | — | | — | | — | | — |
| 15 | Br | 0.002 | F | 0.001 | | — | | — | | — | | — |
| 16 | Al | 0.001 | Mn | 0.001 | | — | | — | | — | | — |
| 17 | Si | 0.001 | | — | | — | | — | | — | | — |
| 18 | Mn | 0.001 | | — | | — | | — | | — | | — |
| | | 100.00 | | 100.00 | | 100.00 | | 100.00 | | 100.00 | | 100.00 |

Column 2 represents, in per cent, the elementary composition of man; Columns 4, 5, 6, and 7 give the average composition of common foods. The data of Column 3 are calculated from the results of Little and represent the elementary composition of the long leaf pine.

and animal organisms; namely, F, Cl, Br, I, Si, Na, Ca, and Mn. These seventeen bioelements are shown in their relative order in Fig. 1, all of them occupying neighboring places.

In addition to these elements there are some apparently essential to certain species of plants and animals, such as Al, Cu, Cs, B, Ba,

TABLE II.

*Distribution of Chemical Elements.*

| Order. | Lithosphere. | | Hydrosphere. | | Atmosphere. | | Meteorites. | |
|---|---|---|---|---|---|---|---|---|
| (1) | (2) *per cent* | | (3) *per cent* | | (4) *per cent* | | (5) *per cent* | |
| 1 | O | 47.33 | O | 85.79 | N | 75.53 | Fe | 72.06 |
| 2 | Si | 27.74 | H | 10.67 | O | 23.02 | O | 10.10 |
| 3 | Al | 7.85 | Cl | 2.07 | Ar | 1.40 | Ni | 6.50 |
| 4 | Fe | 4.50 | Na | 1.14 | H | 0.02 | Si | 5.20 |
| 5 | Ca | 3.47 | Mg | 0.14 | C | 0.01 | Mg | 3.80 |
| 6 | Na | 2.46 | Ca | 0.05 | Kr | 0.01 | S | 0.49 |
| 7 | K | 2.46 | S | 0.05 | Xe | 0.005 | Ca | 0.46 |
| 8 | Mg | 2.24 | K | 0.04 | Remainder | 0.005 | Co | 0.44 |
| 9 | Ti | 0.46 | N | 0.02 | | — | Al | 0.39 |
| 10 | H | 0.22 | Br | 0.01 | | — | Na | 0.17 |
| 11 | C | 0.19 | C | 0.01 | | — | P | 0.14 |
| 12 | P | 0.12 | I | 0.006 | | — | Cr | 0.09 |
| 13 | S | 0.12 | Fe | 0.002 | | — | C | 0.04 |
| 14 | Mn | 0.08 | Remainder | 0.002 | | — | K | 0.04 |
| 15 | Ba | 0.08 | | — | | — | Mn | 0.03 |
| 16 | F | 0.07 | | — | | — | Ti | 0.01 |
| 17 | Cl | 0.06 | | — | | — | Cu | 0.01 |
| 18 | N | 0.02 | | — | | — | Remainder | 0.03 |
| 19 | Sr | 0.02 | | — | | — | | — |
| | Remainder | 0.51 | | — | | — | | — |
| | | 100.00 | | 100.00 | | 100.00 | | 100.00 |

The data of Columns 2, 3, and 4 are taken from Clarke,[4] those of Column 5 from Farrington, O. C., (*Field Museum Nat. History, Publication 120,* 1907; *Publication 151,* 1911), whose report contains analyses of 318 iron and 125 stone meteorites.

Li, Rb, and Zn, while traces of As, Ce, Co, Cr, Mo, Ni, Pb, Ra, Sr, and Ti, have also been reported in specific instances. These elements are marked by a broken circle in Fig. 1 and are also situated close together, with two exceptions.[3]

This summary would be incomplete without a consideration of the abundance of the elements upon our earth. In Table II the data calculated by Clarke[4] are systematically arranged and the order of occurrence in the three spheres (gaseous, liquid, and solid) has been embodied in Fig. 1. It is significant that only the four elements C, N, O, and H occur in all three spheres, while only seven, S, Cl, K, Na, Mg, Ca, and Fe, are predominant in two spheres. Thus the elements of living matter are also the common elements of the earth surface.

Plant organisms contain relatively more of the elements of the soil. Titanium seems to be an exception; although it occurs in traces in nearly all plants,[5] we know little of its physiological functions. Its abundance in celestial bodies, revealed by the spectrum of titanium oxide, would indicate a more important rôle of this element than is known at present.

In conclusion, only 5 per cent of the known elements (namely, C, N, O, and H) forms from 96 to 99 per cent of the biosphere,[6] while about 15 per cent of the elements is invariably or frequently present, another 20 per cent sometimes occurs in traces in certain organisms, and the remaining 60 per cent of the elements is absent under normal conditions; thus from 60 to 80 per cent of the elements (absent and rarely encountered) are of a more or less poisonous character.

[3] These two exceptions, Ce and Pb, would indicate that other members of the carbon group, Zr and Sn, should be found in organisms.

[4] Clarke, F. W., *U. S. Geol. Survey, Bull. 616*, 3rd edition, 1916.

[5] Robinson, W. O., Steinkoenig, L. A., and Miller, C. F., *U. S. Dept. Agric., Bureau of Soils, Bull. 600*, 1917.

[6] These four elements have also been utilized for a system of structure symbols of organic compounds (see Hackh, I. W. D., *Canadian Chem. J.*, 1918, ii, 135; *Science*, 1918, xlviii, 333).

# PHOTOREACTIONS OF PARTIALLY BLINDED WHIP-TAIL SCORPIONS.

By BRADLEY M. PATTEN.

*(From the Laboratory of Histology and Embryology, School of Medicine, Western Reserve University, Cleveland.)*

(Received for publication, December 11, 1918.)

## INTRODUCTION.

The experiments dealt with in this paper were devised to ascertain (1) the relative effectiveness as photoreceptors of the whip-tail scorpion's median eyes, lateral eye groups, and cutaneous sensitive areas, and (2) the effect on orientation produced by symmetrical and by asymmetrical interference with the photoreceptive mechanism.

Most of the animals familiar in the literature on phototropism have light-sensitive mechanisms which consist of a single pair of receptors, or mechanisms in which one of the receptors overshadows the others in effectiveness. The whip-tail scorpion has three pairs of photoreceptors, each of which, acting alone, is capable of bringing about orientation. It offers, therefore, unusually varied possibilities for experiments altering the normal, symmetrical condition of the photoreceptive mechanism.

As a basis for working on partially blinded scorpions, the responses of normal animals were quantitatively determined. The results of this preliminary work have already been published (1), but certain of the more important points may be summarized here. The species of whip-tail used (*Mastigoproctus giganteus* Lucas) was found to be negatively phototropic, and very consistent in its precision of orientation. The method of measuring the normal reactions and the experimental conditions under which the measurements were carried out, were chosen with a view to making even slight changes in reaction clearly recognizable. Responses to known intensities of illumination

were recorded in terms of deflection from an initial direction of locomotion. Deflection amplitudes were measured in degrees by recording the animal's point of emergence from a graduated circle. By making the diameter of the circle relatively small (50 cm.), the rate of attaining orientation, as well as the accuracy of orientation, was made measureable.

The accuracy of orientation exhibited by normal animals when placed between equal, opposed beams of light, was measured as the reaction which would be most readily disturbed by any asymmetrical interference with the receptive mechanism. Deflection amplitudes of animals subjected to anterior and to lateral illumination were measured as being the reactions which would be most extensively changed by symmetrical eliminations of receptors.

The experiments on partially blinded animals reported below were carried out with the same intensity of illumination, and the same method of handling used on normal animals. Change from the normal reaction following the elimination of a photoreceptor can, therefore, be taken as a measurable index of the effectiveness of the receptor prevented from functioning. A series of experiments in which all possible cases of elimination are carried out will show at the same time the relative effectiveness of the different members of the receptive system, and the effect on orientation produced by symmetrical and by asymmetrical interference with the photoreceptors.

### *Apparatus.*

The apparatus used in these experiments was the same as that used in measuring the reactions of normal animals. It is shown in Fig. 1.

Determinations of the illumination delivered by the lights were made with a Bunsen photometer. Each fixed light gave an illumination of 120 candle meters at the center of the observation circle. The intensity of the third light could be varied by moving it to different positions along the axis. The whole apparatus was located in a dark room and in order to reduce reflected light to a minimum, all parts of it except the scales were painted flat black.

*Methods.*

The general external anatomy of the whip-tail scorpion and the more important points concerning its experimental handling have been taken up in a preliminary paper (1). For a detailed morphological consideration of the group to which *Mastigoproctus giganteus*

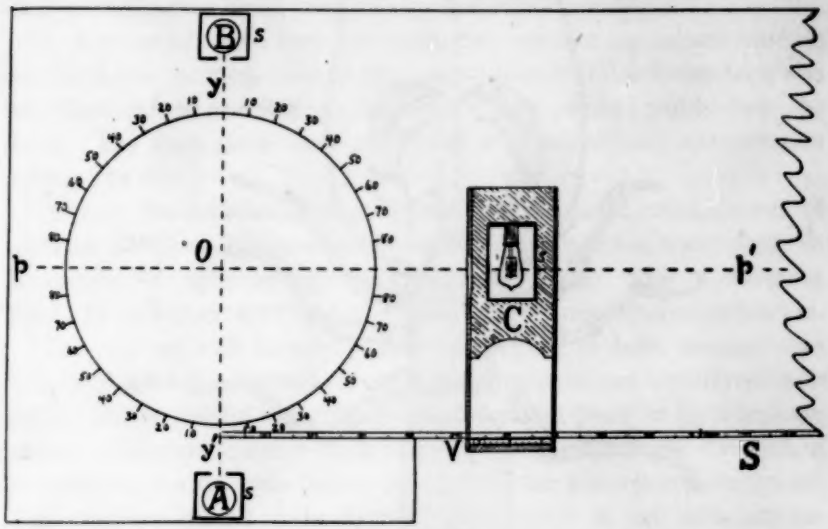



FIG. 1. Plan of apparatus. About *O* as a center a circle 50 cm. in diameter was described and graduated in degrees. This is termed the "observation circle". The axis *pp'* is constructed parallel to the edge of the table and the axis *yy'* perpendicular to *pp'*. On the axis *yy'* two 15 watt Mazda lamps, *A* and B, were set up, each distant 35 cm. from the center of the circle. On the axis *pp'* a similar lamp was mounted on a movable frame, *C*. Affixed at right angles to the base of the frame, *C*, was a scale, *V*, reading as a vernier against the scale, *S*, at the edge of the table. Light-proof cases, *s,s,s*, enclosed each light except for diaphragms 3 × 3 cm. so placed that the beams of light transmitted each centered at *O*.

belongs reference should be made to Börner (2). The sketch of *Mastigoproctus* reproduced in Fig. 2 will serve to give the location of the photoreceptors considered in this paper.

As has already been stated, the method mapped out for securing data on the effectiveness of the various photoreceptors consisted in comparing with the reactions of normal animals, the reactions of ani-

mals in which parts of the light-sensitive system had been prevented from functioning. Various means of eliminating the lateral and median eyes were considered. There seemed no justification, in the

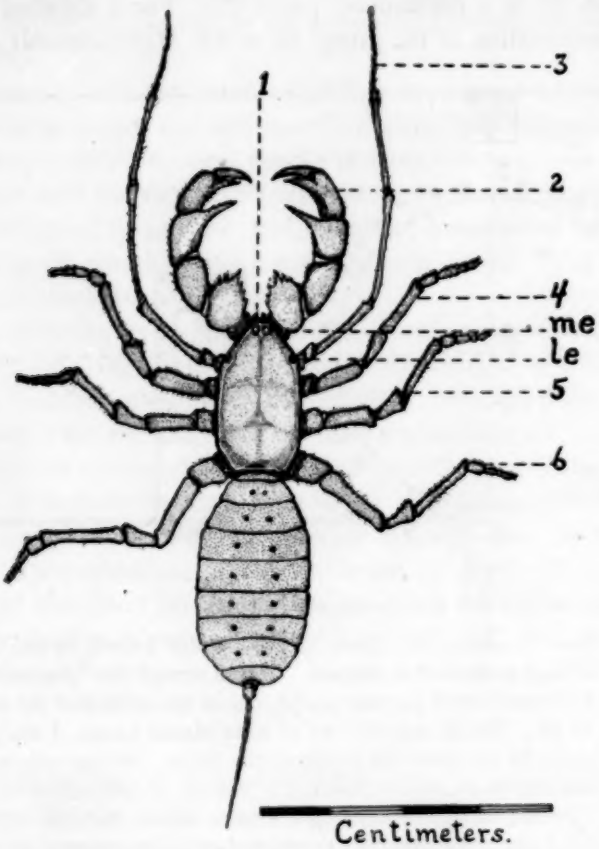



FIG. 2. *Mastigoproctus giganteus* Lucas. *1*, cheliceræ; *2*, pedipalps; *3*, modified anterior legs which serve as feelers; *4*, *5*, *6*, walking legs; *me*, median eyes; *le*, lateral eye group consisting of three eyelets clustered very close together in the form of a miniature triangle.

case of an animal in which the eyes could be effectively covered, for such violent methods as extirpation or cauterization with their possible disturbing after-effects. The following two methods of covering

the eyes were both found to be satisfactory and one or the other was used, without preference, in the series of experiments.

1. Asphaltum varnish was applied with a camel's hair pencil to the surface of the eye and allowed to dry until sticky. A cap of tinfoil cut to fit over the eye was then set in the varnish and allowed to dry in hard. Finally another coat of asphaltum was put over the cap, especial care being used to seal the edges tightly.

2. Rubber adhesive tape was given two coats of asphaltum varnish on the fabric surface. When the second coat had dried, the tape was cut into caps of the desired size, which were pressed firmly over the eyes. The caps were then sealed on with asphaltum varnish and allowed to dry.

Subsequent microscopical examination of cleaned exoskeletons of animals in which the eyes had been capped in these ways gave no indication of light leakage. Furthermore, either type of covering could be removed, leaving the eye uninjured for control experiments.

The first animals handled after the capping of both median eyes and both lateral eye groups showed still a well defined sensitiveness to light. After careful rechecking of the work for possible light leakage around the caps showed that the eyes were effectively covered, a systematic search was begun for cutaneous photosensitive areas.[1] Animals were placed under vertical illumination of just sufficient intensity to make their outlines discernible and searched with a point of intense light obtained by placing a tungsten flashlight bulb in place of the ocular of a compound microscope and converging the rays through the objective (3). As might be expected, the areas of the body covered by thick, heavily pigmented chitin were insensitive to light. This narrowed the search to two localities: the "feeling legs" (see Fig. 2) which had already been demonstrated to be sensitive to touch

[1] The possibility that blinded scorpions might be reacting to the heat of the light was tested in the following way. Animals were illuminated from one side by light of 120 candle meters. On the opposite side, 20 cm. distant from the scorpion, was placed a flatiron emanating heat rays plainly discernible to the back of the hand. The light drove both normal animals and animals with their median and lateral eyes capped toward the iron until they burned their feelers on it. In spite of their crudeness these experiments served to rule out the possibility of a heat reaction playing any part under the conditions of these experiments.

and to chemicals, and the areas of extremely thin and unpigmented chitin around the articulations of the appendages with the sides of the cephalothorax. Careful searching of the feelers with the light spot failed to elicit any reaction from the animal or movement of the feelers. Furthermore when the feelers were cut off several animals, some of which were otherwise normal and some of which had both median and lateral eyes capped, the characteristic orientation to light was in neither case affected.

Attention was then turned to the areas of thin chitin at the sides of the cephalothorax. Searching these areas with the light spot caused the animals to turn away from the illuminated side. The same areas were then rendered opaque by painting with asphaltum varnish.[2] Animals with one side of the cephalothorax blackened, when subjected to bilaterally balanced illumination, showed a very marked deflection toward the blackened side. This deflection was not attributable to mechanical interference with locomotion due to the presence of the varnish, for when stimulated mechanically, the animals did not move in curves. In another series of cases, scorpions with all eyes capped and both sides of the cephalothorax blackened were found to be insensitive to light. Methylene blue preparations of the integument in this region made subsequently showed abundant ganglion cells and nerve endings.

These observations indicated that the analysis of the receptive mechanism must deal with three elements: the median eyes, the lateral eye groups, and the photosensitive areas at the sides of the cephalothorax where the legs articulate with the body. With effective methods of preventing any receptor or group of receptors from functioning, the work resolved itself into series of measurements in which all possible combinations of interference with the photoreceptors should be covered.

[2] The results of this treatment were at first somewhat unsatisfactory because of the mucus thrown out when the areas in question were irritated by the varnish. It was possible, however, by repeated paintings to get a nearly unbroken coat of varnish to adhere. Animals treated in this way had to be used in the desired experiments before the varnish had dried long enough to become brittle.

*Reaction Measurements.*

In all the tests with partially blinded animals, great care was used to reproduce the experimental conditions under which the corresponding tests on normal animals had been made. The method of handling the animals and the methods of measuring and tabulating the reactions were the same as those described for normal animals (1). The details have, therefore, been omitted and the data presented only in the form of tabular and graphic summaries.

For convenience in presentation and consideration, the reaction measurements have been collected in three groups: (1) The reactions to balanced opposed illumination of animals subjected to asymmetrical interference with the photoreceptive mechanism. (2) The reactions to balanced opposed illumination of animals subjected to symmetrical interference with the photoreceptive mechanism. (3) The reactions to lateral and to anterior illumination of animals subjected to symmetrical interference with the photoreceptive mechanism.

*Reactions to Balanced Opposed Illumination of Animals Subjected to Asymmetrical Interference with the Photoreceptive Mechanism.*

In all the experiments on asymmetrical interference, the blackening was carried out on the right side in one-half of the animals, and on the left side in the other half of the individuals used. Tables I and II show the results of measurements made on animals with one lateral eye group capped, and on animals with one side of the cephalothorax blackened. These tables are given in detail partly as illustrations of the method of handling the measurements, but especially to show the consistency and the range of individual variability encountered in typical series of measurements. Qualitatively there can be no doubt as to the significance of the reactions. The approximate consistency of the individual reactions would indicate that averages obtained from the ten trials of an animal express with reasonable quantitative accuracy the value of the reaction.

In the case of the other series of measurements detailed tables have been omitted and only the summary of the results presented. Table III summarizes the measurements made under balanced illumination, on animals subjected to asymmetrical interference with the photoreceptors.

## TABLE I.

*Reactions to Balanced Illumination of Animals with Lateral Eye Group on One Side of Head Covered. Measurements Recorded in Degrees of Deflection from Initial Path of Locomotion Perpendicular to Line Connecting Two Sources of Light.*

| No. of trial. | Animal 6. Right lateral eye group capped. | | Animal 10. Right lateral eye group capped. | | Animal 8. Left lateral eye group capped. | | Animal 9. Left lateral eye group capped. | |
|---|---|---|---|---|---|---|---|---|
| | Deflection to right. | Deflection to left. | Deflection to right. | Deflection to left. | Deflection to right. | Deflection to left. | Deflection to right. | Deflection to left. |
| | *degrees* | *degrees* | *degrees* | *degrees* | *degrees* | *degrees* | *degrees* | *degrees* |
| 1 | 30 | | 35 | | | 22 | | 20 |
| 2 | 50 | | 22 | | | 32 | 0 | 0 |
| 3 | 28 | | 20 | | | 28 | | 40 |
| 4 | 38 | | 45 | | | 30 | | 30 |
| 5 | 40 | | 25 | | | 62 | | 30 |
| 6 | 50 | | 0 | 0 | | 33 | | 20 |
| 7 | 46 | | 22 | | 5 | | | 10 |
| 8 | 20 | | 50 | | | 42 | | 20 |
| 9 | 22 | | 10 | | | 38 | 0 | 0 |
| 10 | 40 | | 30 | | | 45 | | 20 |
| Total... | 364 | | 259 | | 5 | 332 | | 190 |
| Average. | 36.4 to right. | | 25.9 to right. | | 32.7 to left. | | 19 to left. | |

## TABLE II.

*Reactions to Balanced Illumination of Animals with Cutaneous Photosensitive Areas on One Side of Body Blackened. Measurements Recorded in Degrees of Deflection from Initial Path of Locomotion Perpendicular to Line Connecting Two Sources of Light.*

| No. of trial. | Animal 21. Cutaneous sensitive areas on left side blackened. | | Animal 22. Cutaneous sensitive areas on left side blackened. | | Animal 23. Cutaneous sensitive areas on right side blackened. | | Animal 24. Cutaneous sensitive areas on right side blackened. | |
|---|---|---|---|---|---|---|---|---|
| | Deflection to right. | Deflection to left. | Deflection to right. | Deflection to left. | Deflection to right. | Deflection to left. | Deflection to right. | Deflection to left. |
| | *degrees* | *degrees* | *degrees* | *degrees* | *degrees* | *degrees* | *degrees* | *degrees* |
| 1 | | 12 | | 52 | 30 | | 30 | |
| 2 | | 26 | | 30 | 12 | | 20 | |
| 3 | | 12 | | 24 | 40 | | 56 | |
| 4 | | 13 | | 25 | 50 | | 24 | |
| 5 | | 30 | | 22 | 21 | | 34 | |
| 6 | | 28 | | 42 | 52 | | 60 | |
| 7 | | 36 | | 12 | 30 | | 20 | |
| 8 | | 50 | | 18 | 22 | | 20 | |
| 9 | | 48 | | 16 | 28 | | 16 | |
| 10 | | 31 | | 10 | 34 | | 24 | |
| Total... | | 286 | | 251 | 319 | | 304 | |
| Average. | 28.6 to left. | | 25.1 to left. | | 31.9 to right. | | 30.4 to right. | |

TABLE III.

*Summary of Measurements on Asymmetrically Blinded Animals under Balanced Illumination.*

| Operation. | Deflections from initial path of locomotion perpendicular to the line connecting two equally intense sources of light at its mid-point. | | | | |
|---|---|---|---|---|---|
| | Individual averages each based on ten trials. | | | | Average deflection toward less sensitive side. |
| | Animals with right side less sensitive. Deflections to right. | | Animals with left side less sensitive. Deflections to left. | | |
| | Animal 1. | Animal 2. | Animal 1. | Animal 2. | |
| | *degrees* | *degrees* | *degrees* | *degrees* | *degrees* |
| One median eye capped. | 10.5 | 14.1 | 8.2 | 29.7 | 15.6 |
| Both lateral eye groups and median eye on one side capped. | 23.0 | 20.8 | 17.5 | 10.3 | 17.9 |
| Both cutaneous sensitive areas and both lateral eyes blackened and median eye on one side capped. | 21.4 | 31.3 | 23.0 | 32.9 | 27.1 |
| One lateral eye group capped. | 36.4 | 25.9 | 19.0 | 32.7 | 28.5 |
| Cutaneous sensitive areas on one side blackened. | 31.9 | 30.4 | 28.6 | 25.1 | 29.0 |
| Both lateral eye groups and both median eyes capped and cutaneous sensitive areas of one side blackened. | 37.5 | 35.5 | 37.2 | 34.0 | 36.0 |
| One median eye and one lateral eye on same side capped. | 35.7 | 42.3 | 36.6 | 35.9 | 37.6 |
| One median eye and the cutaneous sensitive areas on the same side blackened. | 35.4 | 33.3 | 37.4 | 41.6 | 36.9 |
| One lateral eye and the cutaneous sensitive areas on the same side blackened. | 41.0 | 48.8 | 46.5 | 39.2 | 43.9 |
| All photoreceptors on one side blackened. | Circus movements. | Circus movements. | Circus movements. | Circus movements. | Circus movements. |

The measurements given in Tables I to III are graphically summarized in Fig. 3. The circles represent the observation circle of the apparatus, and the groups of arrows the direction of the lights. The initial position of the animal is indicated by the outline in the center of the circle, and the average path of an animal, based on ten trials, by the radial arrows. On the same figure the average path of travel followed by normal animals, under the same experimental conditions, is indicated by shading. The arrows showing deflections to the right and to the left indicate the courses of animals with the blackening carried out on their right and on their left sides respectively.

In every one of the ten combinations of eliminations in which the photoreceptive mechanism was left functionally asymmetrical, the deflection appears toward the side made less sensitive.

*Reactions to Balanced Opposed Illumination of Animals Subjected to Symmetrical Interference with the Receptive Mechanism.*

As controls to the experiments in which the receptive mechanism was rendered functionally asymmetrical, measurements were made covering the reactions to balanced illumination of animals which had been subjected to bilaterally symmetrical eliminations of their light-sensitive organs.

The measurements are summarized in Table IV and represented graphically in Fig. 4. The manner of representation is the same as that in Fig. 3, the shaded area representing the reaction range of normal animals, and the radial arrows the average paths of the experimental animals. In none of the cases do the reactions of animals with the photosensitive mechanism left functionally symmetrical vary appreciably from the reactions of normal animals. This series of measurements stands in striking contrast to that of Fig. 3 with which it should be compared.

*Reactions to Lateral and to Anterior Illumination of Animals Subjected to Symmetrical Interference with the Photoreceptive Mechanism.*

While symmetrically blinded animals showed little or no variation from the normal when subjected to balanced illumination, under lateral or anterior illumination their attainment of orientation was retarded.

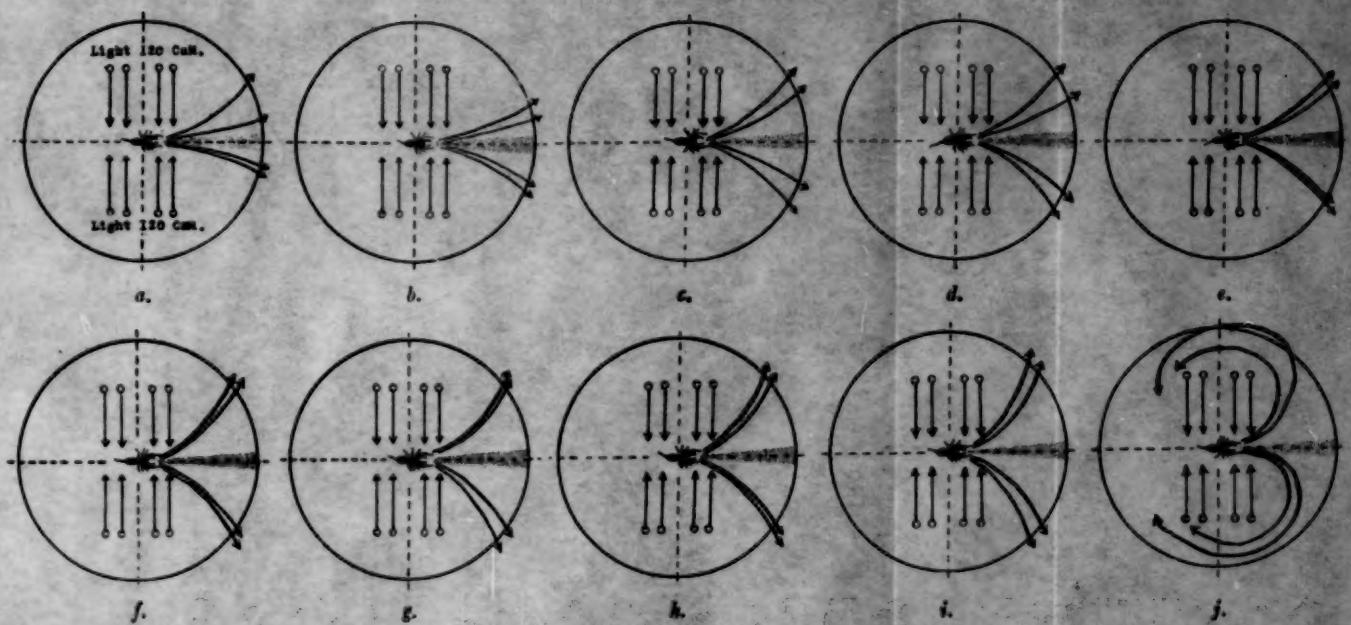
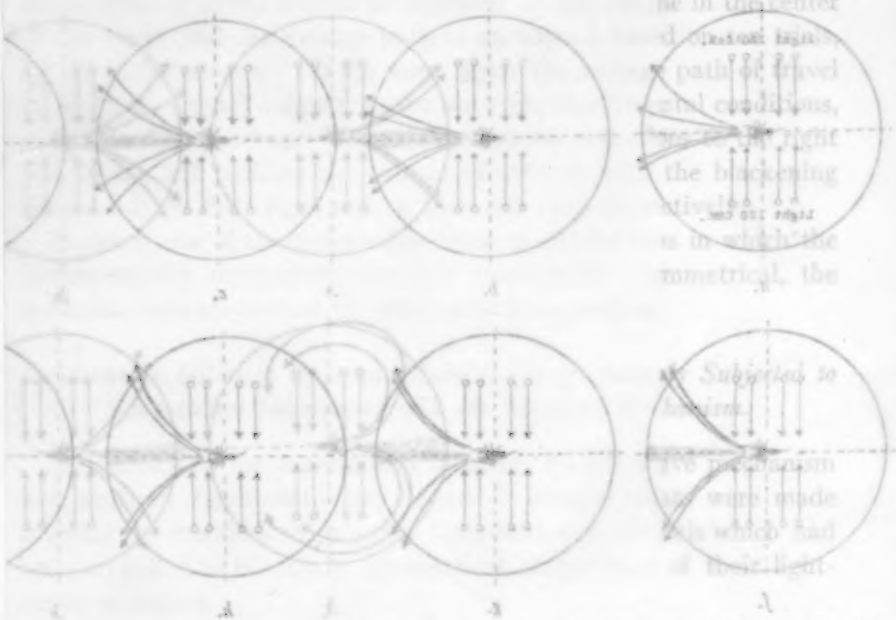



FIG. 3. Graphical summary of reactions to balanced illumination in animals which had been subjected to asymmetrical interference with their photoreceptive mechanism. The circle represents the observation circle of the apparatus; the groups of arrows, the direction of the light beams; the silhouette of the scorpion, the animal's position when subjected to bilateral illumination; the shaded segment, the average reaction range of normal animals; the curved radial arrows, the average paths of experimental animals (based on ten trials). *a*. One median eye capped. *b*. One median eye and both lateral eye groups capped. *c*. One median eye, both lateral eyes, and both cutaneous sensitive areas blackened. *d*. One lateral eye group capped. *e*. Cutaneous sensitive area of one side blackened. *f*. Cutaneous sensitive area of one side blackened and median and lateral eyes on both sides capped. *g*. One median and one lateral eye on the same side capped. *h*. One median eye capped and the cutaneous sensitive area of the same side blackened. *i*. One lateral eye group capped and the cutaneous sensitive area of the same side blackened. *j*. All photoreceptors on one side of the body blackened.



This difference in response in the two cases is due to the difference in the way the animals were brought into the field of light. When subjected to balanced illumination, their initial direction of locomotion was "in orientation" and it was only necessary to maintain it. The symmetrically reduced photosensitive mechanism did this nearly as effectively as the normal. Under lateral or anterior illumination the

TABLE IV.

*Reactions to Balanced Illumination of Animals Previously Subjected to Symmetrical Interference with Photoreceptive Mechanism.*

| Operation. | Deflection from initial path of locomotion perpendicular to line connecting two equally intense sources of light at its mid-point. | | | | |
|---|---|---|---|---|---|
| | Individual averages based on ten trials. | | | | Average path of all animals tested. |
| | Animal 1. | Animal 2. | Animal 3. | Animal 4. | |
| | *degrees* | *degrees* | *degrees* | *degrees* | *degrees* |
| Both median eyes capped. | 0.3 to right. | 0.8 to left. | 0.1 to left. | 0.3 to right. | 0.1 to left. |
| Both lateral eyes capped. | 2.3 to left. | 2.9 to left. | 3.5 to right. | 1.6 to right. | 0.03 to left. |
| Cutaneous sensitive areas on both sides blackened. | 0.2 to left. | 3.9 to right. | 4.3 to right. | 0.6 to right. | 2.1 to right. |
| Both median and both lateral eyes capped. | 4.2 to right. | 4.7 to left. | 3.7 to right. | 0.2 to right. | 0.9 to right. |
| Both lateral eyes capped and cutaneous sensitive areas on both sides blackened. | 2.1 to right. | 4.7 to right. | 4.9 to right. | 3.0 to left. | 2.2 to right. |

animal must *change its direction of locomotion to come into orientation*, and this was accomplished less rapidly in animals with reduced photosensitive areas than in normal individuals. Doubtless if the final orientation were measured, it would vary but little from that of normal animals. Since, however, the reactions are measured by point of emergence from a standard circle, even slight variations in the rate of coming into orientation became apparent, because the method

was based on the degree of orientation attained while the animal was traveling over a standard distance, thus taking into account the time factor.

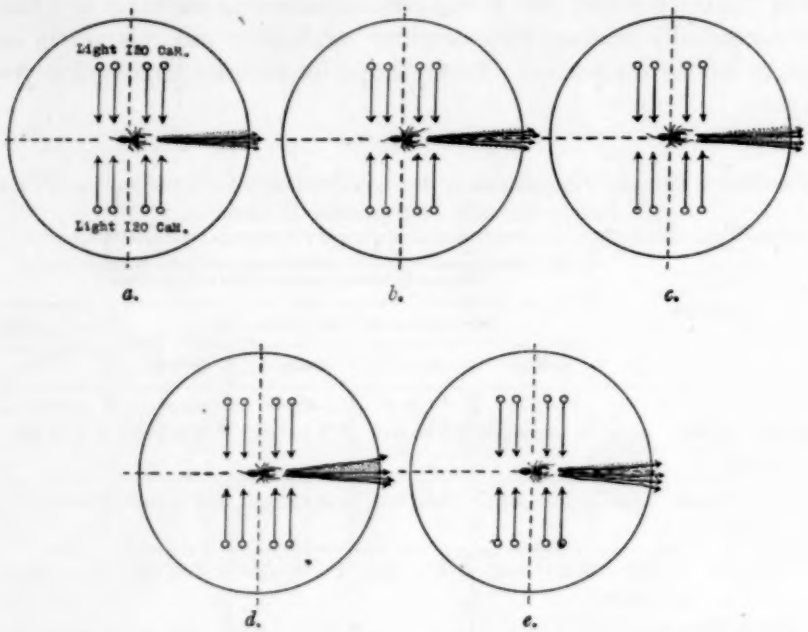



FIG. 4. Graphical summary of reactions to balanced illumination in animals which had been subjected to symmetrical interference with their photoreceptive mechanism. The circle represents the observation circle of the apparatus; the groups of arrows, the direction of the light beams; the silhouette of the scorpion, the animal's position when subjected to bilateral illumination; the shaded segment, the average path of normal animals; the curved radial arrows, the average paths of experimental animals (based on ten trials). *a*. Animals with both median eyes capped. *b*. Both lateral eye groups capped. *c*. Cutaneous sensitive areas on both sides blackened. *d*. Both lateral and both median eyes capped. *e*. Both lateral eyes and the cutaneous sensitive areas on both sides blackened.

The reaction measurements made on symmetrically blinded animals subjected to lateral illumination are collected in Table V and in Fig. 5. The solid arrows represent the average path followed by the partially blinded animals, the dotted arrows the average reaction of normal animals under the same conditions of illumination.

TABLE V.

*Reactions to Lateral Illumination of Animals Previously Subjected to Symmetrical Interference with Photoreceptive Mechanism.*

| Operation. | Deflection from initial path of locomotion at right angles across the beam of light. | | | | |
|---|---|---|---|---|---|
| | Individual averages based on ten trials. | | | | Average of all animals tested. |
| | Animal 1. | Animal 2. | Animal 3. | Animal 4. | |
| | *degrees* | *degrees* | *degrees* | *degrees* | *degrees* |
| Both median eyes capped. | 61.3 | 61.9 | 58.3 | 55.2 | 59.2 |
| Both lateral eyes capped. | 43.5 | 50.5 | 60.3 | 67.4 | 55.4 |
| Cutaneous sensitive areas of both sides blackened. | 60.1 | 48.1 | 38.9 | 45.1 | 48.1 |
| Both median and both lateral eyes capped. | 52.5 | 41.3 | 39.5 | 44.2 | 44.4 |
| Both lateral eyes capped and cutaneous sensitive areas of both sides blackened. | 42.6 | 30.4 | 44.6 | 23.8 | 35.4 |
| All photoreceptors blackened. | +8.8* | 6.3 | 4.6 | 2.0 | 1.0 |

The plus sign indicates that this deflection was *toward* the light. All other deflections in the table are away from the light, or negative.

TABLE VI.

*Reactions to Anterior Illumination of Animals Previously Subjected to Symmetrical Interference with Photoreceptive Mechanism.*

| Operation. | Deflection from initial path of locomotion directly toward source of light. | | | | |
|---|---|---|---|---|---|
| | Individual averages based on ten trials. | | | | Average of all animals tested. |
| | Animal 1. | Animal 2. | Animal 3. | Animal 4. | |
| | *degrees* | *degrees* | *degrees* | *degrees* | *degrees* |
| Both median eyes capped. | 117.3 | 123.5 | 136.6 | 132.6 | 127.5 |
| Both lateral eyes capped. | 124.9 | 128.8 | 127.8 | 140.9 | 130.6 |
| Cutaneous sensitive areas of both sides blackened. | 120.2 | 121.0 | 101.2 | 100.4 | 110.7 |
| Both lateral and both median eyes capped. | 118.3 | 116.0 | 108.3 | 115.8 | 114.6 |
| Both lateral eyes capped and cutaneous sensitive areas on both sides blackened. | 91.3 | 88.8 | 98.4 | 101.0 | 94.9 |

Table VI and Fig. 6 show the reactions of symmetrically blinded animals to anterior illumination. The experimental conditions under which these measurements were made are essentially similar to those

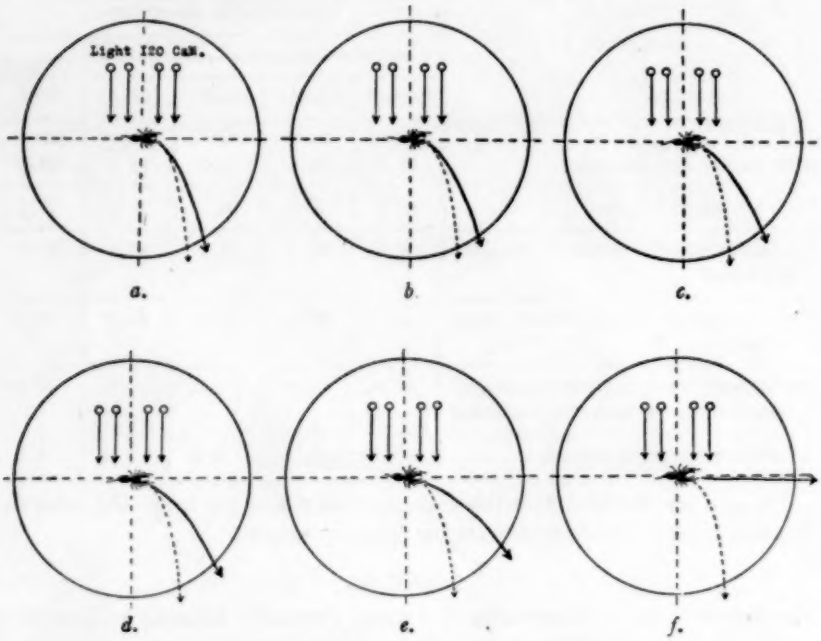



FIG. 5. Reactions to lateral illumination in animals which had been subjected to symmetrical interference with their photoreceptive mechanism. The circle represents the observation circle of the apparatus; the group of arrows, the direction of the light beam; the silhouette of the scorpion, the animal's position when subjected to lateral illumination; the dotted arrow, the average reaction of normal animals; the solid arrow, the average reaction of the experimental animals. *a*. Animals with both median eyes capped. *b*. Animals with both lateral eye groups capped. *c*. Cutaneous sensitive areas on both sides blackened. *d*. Both median and both lateral eyes capped. *e*. Both cutaneous sensitive areas and both lateral eyes blackened. *f*. All photoreceptors blackened.

set up when lateral illumination was used. The difference lies only in the greater deflection an animal must make to come into orientation when subjected to anterior illumination. A comparison of Figs.

5 and 6 shows that the reactions under lateral and under anterior illumination are qualitatively the same.

Quantitative comparison based on the percentage reduction of the normal reaction in each case shows a correspondence which is

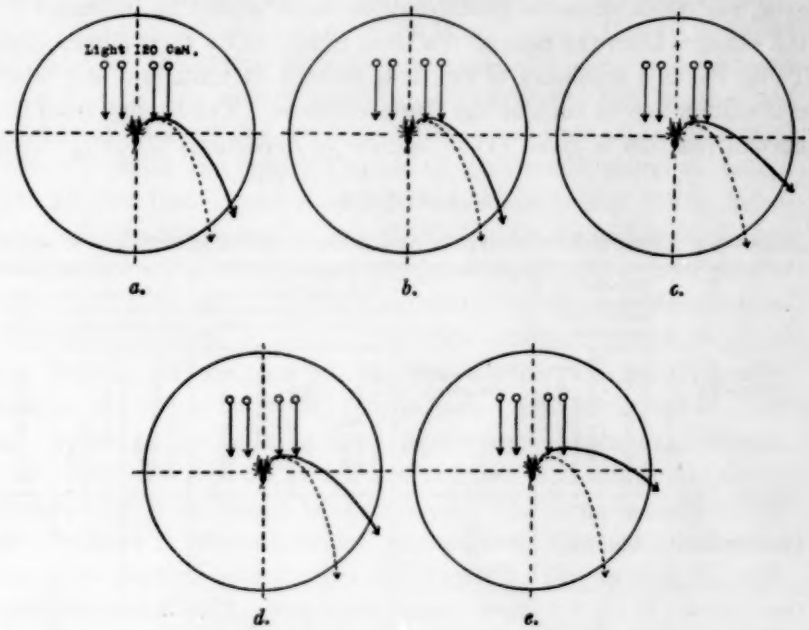



FIG. 6. Reactions to anterior illumination in animals which had been subjected to symmetrical interference with their photoreceptive mechanism. The circle represents the observation circle of the apparatus; the group of arrows, the direction of the light beam; the silhouette of the scorpion, the animal's position when subjected to anterior illumination; the dotted arrow, the average reaction of normal animals; the solid arrow, the average reaction of the experimental animals. *a*. Animals with both median eyes capped. *b*. Animals with both lateral eyes capped. *c*. Cutaneous sensitive areas on both sides blackened. *d*. Both median and both lateral eyes capped. *e*. Both cutaneous sensitive areas and both lateral eyes blackened.

within the limits of experimental error, when the differences in effective illumination due to the anatomical position of the receptors is taken into consideration (see below).

## DISCUSSION.

### *Relative Effectiveness of Photoreceptors.*

The relative effectiveness of the lateral eye groups, the median eyes, and the cutaneous photosensitive areas should be indicated by the changes from the normal reactions produced by their elimination. Table VII is a summary of the data secured by unilateral and bilateral elimination of each of the photoreceptors. The change from the normal reaction is given (1) in degrees of deflection; (2) in per cent

TABLE VII.

*Summary of Effects of Eliminating Different Members of Photoreceptive Mechanism.*

| Organ eliminated. | Unbalance as measured under bilateral illumination. | | Pair of organs eliminated. | Reduction from normal reaction as measured under lateral illumination. | | | Reduction from normal reaction as measured under anterior illumination. | | |
|---|---|---|---|---|---|---|---|---|---|
| | Deflection toward blackened side. | Relative values of deflections toward the blackened side. | | Measured in degrees of deflection. | In per cent of normal reaction. | Relative values. | Measured in degrees of deflection. | In per cent of normal reaction. | Relative values. |
| | *degrees* | | | | | | | | |
| One median eye. | 15.6 | 1.0 | Both median eyes. | 6.6 | 10 | 1.0 | 12.9 | 9.2 | 1.0 |
| One lateral eye. | 28.5 | 1.8 | Both lateral eyes. | 10.4 | 15.9 | 1.6 | 9.8 | 7.0 | 0.8 |
| One cutaneous sensitive area. | 29.0 | 1.9 | Both cutaneous sensitive areas. | 17.7 | 26.8 | 2.6 | 29.7 | 21.2 | 2.3 |

of the normal reaction; and (3) in relative values calculated by taking the change produced by elimination of the median eyes as unity.

There are certain apparent inconsistencies in the data of Table VII that require consideration before we attempt to draw any conclusions as to the relative effectiveness of the photoreceptors.

There is good agreement between the reductions in normal reaction produced by the elimination of the median eyes under lateral and under anterior illumination, the value of the reduction from normal being 10 per cent under lateral illumination and 9.2 per cent under

anterior illumination. The changes from the normal reaction induced by elimination of the lateral eyes, however, do not show the same consistency, the values being 15.9 per cent reduction of the normal reaction under lateral illumination, and 7 per cent reduction under anterior illumination. This discrepancy is, I believe, attributable to the anatomical location of the lateral eyes. Their position at the sides of the cephalothorax, surrounded by heavily pigmented chitin, is such that a very small proportion of the light in the field would be effective on the retinulæ of the lateral eyes when the animal is directly facing the light. The lateral eyes would, therefore, be operating below their capacity during a considerable part of the deflection made by animals headed into the light. On the other hand, they would be operating at maximum efficiency during the greater part of the deflection made by animals subjected to lateral illumination. The median eyes are so placed that they receive approximately the same amount of effective illumination whether the animal is subjected to anterior or to lateral illumination. The conditions of shading, involved in the initial positions of the animal account, therefore, for the discrepancy in the two sets of reaction measurements made on animals with their lateral eyes covered. The same considerations would indicate that measurements under lateral illumination express more correctly than experiments under anterior illumination the relative effectiveness of the photoreceptors.

Another apparent inconsistency in the data of Table VII is revealed by adding the reductions in deflection produced by the separate elimination of each receptor. Taking as a basis the measurements made under lateral illumination, the reductions in deflection caused by the separate elimination of the three photoreceptors total to a value approximately only one-half the normal reaction; yet when the three receptors are simultaneously eliminated the animals are practically insensitive to light (Table V and Fig. 5). It is possible to attribute these conditions to a compensatory increase in activity on the part of the unblackened receptors. While this may be in part responsible, I believe the greater part, at least, of the discrepancy can be otherwise explained. In each of the series of measurements in which some part of the photoreceptive mechanism is prevented from functioning, the reduction in deflection is measured at what might be called the

upper end of the deflection range. It has been shown (1) that the increase in illumination necessary to produce an initial deflection of definite value is less than the increase in illumination necessary to produce an increase in deflection of like value. The data collected were not sufficiently extensive to justify the statement that the increases in deflection followed mathematically the Weber-Fechner law, but the curve of increase in deflection with increasing lateral illumination is certainly of that general type. Applied to the data under consideration, this means that the first 10° of deflection are more readily induced than the deflection from 10° to 20° and so on. A reduction in deflection to 50° from a normal of 60°, following interference with receptors, does not indicate therefore that the receptive mechanism has been reduced by one-sixth in efficiency. On the contrary, it would require a reduction of considerably more than one-sixth in photoreceptive effectiveness to reduce by one-sixth the normal reaction.

While it is not possible, in the light of the above considerations, to compute the effectiveness of the different receptors on the basis of the percentage reduction of normal reactions produced by their elimination, their relative effectiveness may, nevertheless, be deduced from the available data. All the induced reductions in reaction, because of the method of measurement employed, fall in the same part of the deflection range. They can, therefore, justifiably be compared with each other. This comparison has been worked out in Table VII by taking the effect of the elimination of the median eyes as unity and comparing with it the effect produced by elimination of the other receptors. By averaging[3] the values thus obtained under the three different conditions of illumination used, we can approximate the relative effectiveness of the photoreceptors as median eyes : lateral eyes : cutaneous areas :: 1 : 1.6 : 2.2.

[3] In computing the averages the responses to anterior illumination made by animals with their lateral eyes capped were not given equal weight with those made under lateral illumination. The reasons for regarding the experiments under anterior illumination as less accurate as far as indicating the effectiveness of the lateral eyes is concerned, are given in the text.

*Effect on Orientation Produced by Symmetrical and by Asymmetrical Interference with the Photoreceptive Mechanism.*

Small changes in the tonus of the muscles of locomotion are frequently difficult to detect and always difficult to handle quantitatively in an animal which is not moving. An animal's locomotion, however, is a direct resultant of muscular activities and any difference in the tonus or in the vigor of contraction of the muscles on opposite sides of the body is at once evidenced by a deflection in the path of advance. Small differences in contraction are summated in continued locomotion. The measurement of deflections in the path of travel is, therefore, a convenient and accurate method of dealing quantitatively with the activities of locomotor muscles.

Unbalanced muscular reactions as indicated by a curved path of locomotion have been observed to follow unilateral blinding in both positively and negatively phototropic animals. The earlier observations were qualitative only, more recently the reactions have been quantitatively handled. In all the cases the evidence is in accord with Loeb's muscle tension theory of orientation, positively phototropic forms moving in curves with their uninjured side inward, and negative forms in curves with their blackened side inward (4).

The reaction measurements made in terms of angular deflection on partially blinded whip-tail scorpions fall entirely in line with observations on other animals. But because the scorpion has three pairs of photoreceptors, each of which can be eliminated separately, the series of measurements which can be made on it is peculiarly extensive and interesting. It is possible to eliminate on one side of the body the median eye, the lateral eye, or the cutaneous sensitive areas; or any two receptors; or all three receptors. By making eliminations on both sides of the body, the series of cases may be still further extended. In these experiments measurements were made, under bilaterally balanced illumination, on animals in ten different conditions of asymmetry. The results are collected in Table III. The graphical summary (Fig. 3) shows clearly the unerring consistency with which all the animals were deflected toward their less sensitive side. All the deflections are to be regarded as having the same significance as circus movements. In most of the deflections the radius of the curved

path followed by the animals is greater than the radius of the observation circle of the apparatus. When the induced asymmetry is extreme, typical circus movements of small radius result. The point of emergence of the trails of larger radius on the standard observation circle, gives an index of the curvature of the arc of locomotion in quantitative terms which are comparable with the other reaction measurements. The constant curving of the path of locomotion in animals which are asymmetrically sensitive stands in sharp contrast to the locomotion of normal animals under bilaterally unequal illumination. In the latter case the path of locomotion is straight once the direction of crawling becomes such that the inequality in illumination in the field is equalized in its effectiveness on the photosensitive areas of the animal by greater exposure of the sensitive areas on the side of less intense illumination, and lesser exposure of the sensitive areas on the side of more intense illumination (5). Asymmetrically sensitive animals under vertical illumination continue in a curving path of locomotion because it is impossible through changes in axial position to equalize bilaterally the effective illumination. Under equal and opposed horizontal illumination slight bilateral inequalities in sensitiveness may be compensated for by the assumption of an axial position inclined toward the less intense light. When the asymmetry of sensitiveness is made extreme the effective illumination cannot thus be brought into bilateral equilibrium and the curved path of locomotion is continued.

A feature deserving further comment is the way in which the amplitude of the deflections increases as the degree of asymmetry in sensitiveness is increased. The cumulative effect produced by blackening more than one receptor on the same side of the head is obvious from the figures. Less apparent but equally significant is the fact that the unbalance in reaction is greater when all the receptors except a given one are eliminated, than when the same receptor is the only one covered. For example, the deflection induced when the median eye on one side is capped averages 15.6°; when both lateral eyes, the cutaneous sensitive areas on both sides, and the median eye on one side are blackened (leaving functional only one median eye), the deflection averages 27.1° (see Table III). The unbalanced factor in each experiment is a median eye, but when the median eye alone is elim-

inated the balance of the receptive mechanism as a whole is less disturbed than when the median eye on one side is the only functional receptor.

It is not pertinent here to enter into a detailed comparison of these results on the scorpion with the experiments of Garrey (6) on the positively phototropic robber fly, but the complete agreement of the two series of observations made by different methods, on animals having opposite signs of phototropism, is too striking to pass without comment.

Further evidence that balanced reactions depend on the functional symmetry of the receptive mechanism is furnished by the measurements made on animals in which the photoreceptors had been symmetrically interfered with. As in the case of asymmetrically sensitive scorpions, the symmetrically blinded animals were brought into the field of equal opposed lights "in orientation" (*i.e.*, with their plane of symmetry perpendicular to the line connecting the sources of light). No matter what eliminations were made, so long as the photoreceptive mechanism was left in a functionally symmetrical condition it sufficed to maintain[4] a balanced response which was accurate within the limits of variability exhibited by normal animals under the same conditions of illumination (Table IV and Fig. 4).

Observations already published by many different investigators have covered nearly every phase of the correlation existing between bilaterally balanced excitation of photoreceptors and balanced locomotor responses. A constantly increasing accumulation of experimental evidence indicates that the attaining and maintaining of orientation to light depends, as postulated in Loeb's muscle tension theory, on the transmission to the muscles of locomotion of impulses which are proportional bilaterally to the excitation of symmetrically located photoreceptors.

The results of the experiments described above indicate that the muscle tension theory applies to the complex receptive mechanism of

[4] It has already been pointed out that when brought into the field of light out of orientation (as in the experiments under anterior and lateral illumination) symmetrically blinded animals exhibited a retardation in their rate of coming to a new direction of orientation.

the whip-tail scorpion as well as to animals with a single pair of receptors. The evidence may be summarized as follows:

In normal scorpions bilateral equilibrium in excitation results invariably in bilaterally balanced muscular responses and a straight path of locomotion.

When the receptive mechanism is reduced but left functionally symmetrical, the locomotor responses remain balanced. Animals in five different symmetrical conditions of reduced sensitiveness showed no unbalance in reaction although the rate of attaining orientation was reduced in proportion to the extent of the interference with the photoreceptive mechanism.

When the receptive system is subjected to eliminations which leave it in an asymmetrical condition, unbalanced locomotor responses invariably follow, resulting in a curved path of locomotion with the less sensitive side of the animal on the inside of the curve. In a series of ten different conditions of asymmetrical sensitiveness the degree of unbalance in locomotion was proportional to the extent of asymmetry which had been produced in the receptive apparatus.

In their effect on orientation, the three pairs of receptors are completely coordinated, the excitation of the organs on the same side of the head being summated in transmission to the associated muscles of locomotion.

Balanced muscular reactions with a persistently straight path of locomotion depend on bringing the excitation of the receptive mechanism functional at the time into bilateral equilibrium.

## SUMMARY.

The experiments dealt with in this paper were devised to ascertain (1) the relative effectiveness as photoreceptors of the whip-tail scorpion's median eyes, lateral eye groups, and cutaneous sensitive areas, and (2) the effect on orientation produced by symmetrical and by asymmetrical interference with the photoreceptive mechanism.

Each of the receptors was eliminated unilaterally and bilaterally, singly and in combinations with other receptors. In all, sixteen different abnormal conditions of the photoreceptive apparatus were produced.

The reactions of animals thus partially blinded were measured in terms of angular deflection from an initial path of locomotion. Measurements obtained under anterior, lateral, and bilaterally balanced illumination were compared with measurements made on normal animals under the same conditions of illumination. The change from the normal reaction induced by covering a photoreceptor was taken as an index of the effectiveness of the receptor prevented from functioning.

By comparing the values of the changes from normal reactions produced by the elimination of the several receptors, their relative effectiveness is approximated as median eyes : lateral eyes : cutaneous sensitive areas :: 1 : 1.6 : 2.2.

All animals in which the receptive mechanism was rendered functionally asymmetrical exhibited, when subjected to bilaterally balanced illumination, deflections toward the side which had been made less sensitive. In a series of measurements made on animals in ten different conditions of asymmetry the amplitudes of the deflections were proportional to the degree of unbalance which had been produced in the photosensitive mechanism.

Animals in which the receptive mechanism was reduced but left in a symmetrical condition maintained an undisturbed balance of reaction when subjected to equal, opposed lights. Under lateral or anterior illumination the rate of attaining a new direction of orientation was reduced in proportion to the extent of the interference with the receptive mechanism.

The reactions of symmetrically and asymmetrically blinded scorpions indicate that orientation is attained and maintained by a transmission of impulses to the muscles of locomotion which is proportional bilaterally to the excitation of the symmetrically located photoreceptors.

In their effect on orientation the three pairs of receptors are completely coordinated. Orientation depends upon bringing the excitation of the receptive mechanism as a whole into bilateral equilibrium.

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# A STUDY OF THE ACTION OF ACID AND ALKALI ON GLUTEN.

By L. J. HENDERSON, EDWIN J. COHN,* P. H. CATHCART,* J. D. WACHMAN,* AND W. O. FENN.*

*(From the Wolcott Gibbs Memorial Laboratory, Harvard University, Cambridge, in Collaboration with the Division of Food and Nutrition, Medical Department, U. S. Army.)*

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The problem of the acid-base equilibrium in systems containing protein substances and electrolytes has often been obscured by superficial interpretations of experimental results and, in recent investigations, by undue emphasis upon the colloidal aspects of the phenomena. Nevertheless, it has always remained certain that proteins, as amphoteric substances, must under all circumstances, except when pure at the isoelectric point, combine chemically with acids or bases. Moreover, the simple relationships involved in such equilibria are the most important factors in determining the properties of the systems. This conception of the behavior of protein systems has recently been reaffirmed and amplified by Sörensen and his associates,[1] and by Loeb.[2]

The studies which are reported in the present paper point in this same direction. Our experiments have been made with gluten, in some respects not the most favorable material for investigation, because it contains two distinct proteins, because it has not been highly purified from electrolytes, and because it possesses marked colloidal properties. In spite of these obstacles, which must therefore be less important than they seem, the results indicate a simple chemical interpretation of the phenomena.

This research was undertaken under the pressure of war time practical interests, for the purpose of explaining the technology of bread

* Lieutenant, Sanitary Corps, U. S. Army.

[1] Sörensen and others, *Compt. rend. trav. Lab. Carlsberg*, 1917, xii.

[2] Loeb, J., *J. Biol. Chem.*, 1917, xxxi, 343; 1918, xxxiii, 531; 1918, xxxiv, 77, 395, 489; 1918, xxxv, 497; *J. Gen. Physiol.*, 1918–19, i, 39.

making. Accordingly the measurements have in some respects not been pushed to the highest possible accuracy, nor has every part of the question been carefully surveyed.

## I.

The first part of the work consisted of measurements, with the concentration cell, of the hydrogen ion concentration of solutions which had been in contact with powdered gluten.

The data in Table I show that, as a first approximation, the hydrogen ion concentration in such systems is determined by the ratio of gluten to acid, or gluten to base. In the acid systems this is quite clear; in the alkaline systems, where unknown difficulties have interfered with the accuracy of the measurements, it is perhaps somewhat less apparent. But, in view of the fact that under the circumstances equilibrium can hardly be perfectly attained, and on account of the numerous other difficulties which are inherent in work of this kind, the evidence seems satisfactory.

This result accords with the work of Sörensen. But the system that we have studied contains large amounts of protein in a discrete phase, and frequently also large amounts dispersed throughout the aqueous solution, and therefore possesses a very high degree of complexity. It is on this account especially important that the simple relationship still holds good. Unpublished measurements upon purified glutenin and gliadin further confirm this observation. Therefore we venture to draw the conclusion that in systems containing gluten and acids or bases the formation of salts, in accordance with the requirements of the mass law, is the fundamental phenomenon. Here, as in all cases where a weak acid or base in excess is in equilibrium with a strong base or acid, the hydrogen ion concentration is dependent upon the ratio of acid to salt or of salt to base, as the case may be. This relationship is of course only approximate, less true in the neighborhood of the isoelectric point. Compared with the case of simple substances, it is modified in a definite and characteristic manner by the polybasic and polyacid character of the amphoteric protein. It is not, however, seriously modified by the heterogenity of the system or by the colloidal character of the protein. This subject will be discussed theoretically in a later paper.

TABLE I.

*Hydrogen Ion Concentration of Solutions of NaOH and HCl containing Gluten in Different Concentrations.*

| A | | | | B | | | |
|---|---|---|---|---|---|---|---|
| Gluten in 100 cc. solution. | Concentration of HCl. | $\left(R \frac{HCl}{gluten}\right)$ | pH of HCl containing gluten. | Gluten in 100 cc. solution. | Concentration of NaOH. | $R \left(\frac{NaOH}{gluten}\right)$ | pH of NaOH containing gluten. |
| *gm.* | *N* | | | *gm.* | *N* | | |
| 1 | 0.01 | 10 | 2.23 | 8 | 0.00025 | 0.031 | 5.91 |
| 1 | 0.005 | 5 | 2.68 | 2 | 0.00012 | 0.062 | 5.97 |
| 2 | 0.01 | | 2.46 | 4 | 0.00025 | | 6.05 |
| | | | | 8 | 0.0005 | | 6.06 |
| 2 | 0.005 | 2.5 | 3.32 | | | | |
| 4 | 0.01 | | 3.12 | 1 | 0.00012 | 0.125 | 6.19 |
| | | | | 2 | 0.00025 | | 6.39 |
| 1 | 0.002 | 2.0 | 3.78 | 4 | 0.0005 | | 6.41 |
| | | | | 8 | 0.001 | | 6.22 |
| 4 | 0.005 | 1.5 | 4.16 | | | | |
| | | | | 1 | 0.00025 | 0.25 | 6.87 |
| 1 | 0.001 | 1.0 | 4.43 | 2 | 0.0005 | | 6.87 |
| 2 | 0.002 | | 4.52 | 4 | 0.001 | | 6.82 |
| | | | | 8 | 0.002 | | 6.69 |
| 1 | 0.0005 | 0.5 | 4.93 | | | | |
| 2 | 0.001 | | 4.98 | 1 | 0.0005 | 0.5 | 7.72 |
| 4 | 0.002 | | 5.00 | 2 | 0.001 | | 7.53 |
| | | | | 4 | 0.002 | | 7.45 |
| 2 | 0.0005 | 0.25 | 5.27 | 8 | 0.004 | | 7.15 |
| 4 | 0.001 | | 5.34 | | | | |
| 8 | 0.002 | | 5.34 | 1 | 0.001 | 1.0 | 9.63 |
| | | | | 2 | 0.002 | | 9.37 |
| 4 | 0.0005 | 0.125 | 5.43 | 4 | 0.004 | | 9.28 |
| 8 | 0.001 | | 5.55 | | | | |
| | | | | 1 | 0.002 | 2.0 | 10.08 |
| 8 | 0.0005 | 0.062 | 5.64 | 2 | 0.004 | | 10.70 |
| 1 | 0 | 0 | 5.64 | 4 | 0.01 | 2.5 | 10.76 |
| 2 | | | 5.69 | | | | |
| 4 | | | 5.73 | 1 | 0.004 | 4.0 | 11.26 |
| 8 | | | 5.69 | | | | |

## II.

Further analysis of the system is made possible by measurements of the electrical conductivity of the aqueous phase.

In Table II, which takes account only of the acid systems, because of the greater consistency of the measurements, the concentration of free hydrochloric acid is calculated from the measurements of hydrogen ion concentration; that part of the conductivity due to hydrochloric acid is next calculated, and this is then subtracted from the observed con-

TABLE II.

| Gluten in 100 cc. solution. | HCl original concentration. | pH | $\frac{+}{(H)}$ | Combined HCl. | Conductivity. | Conductivity of free HCl. | Salt conductivity. | Corrected salt conductivity. | R | X |
|---|---|---|---|---|---|---|---|---|---|---|
| gm. | N | | N | | | | | | | |
| 1.0 | 0.01 | 2.23 | 0.0059 | 0.0041 | 2,851 | 2,450 | 401 | 353 | 86 | 150 |
| | 0.005 | 2.68 | 0.00209 | 0.00291 | 1,142 | 870 | 272 | 224 | 77 | 75 |
| | 0.002 | 2.78 | 0.000166 | 0.00184 | 255 | 69 | 186 | 138 | 75 | 45 |
| | 0.001 | 4.43 | 0.000037 | 0.000965 | 132 | 15.4 | 116.6 | 68 | 71 | 20 |
| | 0.0005 | 4.93 | 0.0000118 | 0.000490 | 89 | 4.9 | 84.1 | 36 | (73) | 10 |
| | 0.0000 | 5.64 | | | 48.5 | | | | | |
| 2.0 | 0.01 | 2.46 | 0.00347 | 0.00653 | 2,133 | 1,480 | 653 | 565 | 86 | 240 |
| | 0.005 | 3.32 | 0.00048 | 0.00452 | 605 | 199 | 406 | 318 | 70 | 90 |
| | 0.002 | 4.52 | 0.0000302 | 0.00197 | 234 | 12.6 | 221.4 | 133 | 67 | 35 |
| | 0.001 | 4.98 | 0.0000105 | 0.000991 | 132 | 4.35 | 127.6 | 39 | (39) | |
| | 0.0005 | 5.27 | 0.0000054 | 0.000497 | 137 | 2.24 | 134.8 | 46 | (93) | |
| | 0.0000 | 5.69 | | | 88.5 | | | | | |
| 4.0 | 0.01 | 3.12 | 0.00076 | 0.00924 | 1,113 | 316 | 797 | 649 | 70 | 200 |
| | 0.005 | 4.16 | 0.000069 | 0.00493 | 491 | 28.6 | 462.4 | 314 | 64 | 70 |
| | 0.002 | 5.00 | 0.000010 | 0.00199 | 276 | 4.15 | 271.8 | 123 | 62 | 25 |
| | 0.001 | 5.34 | 0.0000046 | 0.000997 | 270 | 1.91 | 268.1 | 120 | (120) | |
| | 0.0005 | 5.43 | 0.0000037 | 0.000498 | 219 | 1.54 | 217.5 | 69 | (138) | |
| | 0.0000 | 5.73 | | | 148.5 | | | | | |
| 8.0 | 0.002 | 5.34 | 0.0000046 | 0.001997 | 391 | 19.1 | 389.1 | 109 | 55 | 10 |
| | 0.001 | 5.55 | 0.0000028 | 0.000999 | 306 | 11.6 | 304.8 | 25 | (25) | |
| | 0.0005 | 5.64 | 0.0000023 | 0.0004997 | 278 | 09.6 | 277.0 | −3 | | |
| | 0.0000 | 5.69 | | | 280 | | | | | |

ductivity. The remainder, given in Table II as "salt conductivity," represents the effect of all other ions than those which may be hypothetically attributed to free hydrochloric acid. From this remainder the conductivity of the system in the absence of hydrochloric acid is subtracted and the result is called "corrected salt conductivity." It represents the effect of the action of the acid, chemically or other-

wise, to increase conductivity, aside from the direct effect of the ions arising from those molecules of acid which remain free in the solution. Finally this value is compared with the total amount of acid which has disappeared from the solution, and which may therefore be held responsible, directly or indirectly, for this action. This comparison is expressed in the form of a ratio designated in Table II as R.

It will be seen that there is a rough constancy of the ratio of corrected salt conductivity to combined acid. As might have been expected, this is not true in the lower ranges of acidity where the experimental errors, presumably of constant magnitude, are large in proportion to the effects of the acid added.

Disregarding the systems where the quantity of acid is small, it may also be seen that the numbers vary in a regular manner. The greater the hydrogen ion concentration, the greater are the values of R (Table III).

TABLE III.
*Values of R.*

| pH | Gluten in 100 cc. | | | |
|---|---|---|---|---|
| | 1 gm. | 2 gm. | 4 gm. | 8 gm. |
| 2.2 | 86 | | | |
| 2.6 | 77 | 86 | | |
| 3.2 | | 70 | 70 | |
| 3.8 | 75 | | | |
| 4.2 | | | 64 | |
| 4.5 | 71 | 67 | | |
| 5.0 | | | 62 | |
| 5.3 | | | | 55 |

In order to investigate this subject, we may provisionally assume that, however it comes about, the effect upon the conductivity of the formation of a given amount of protein chloride is constant. Of course this can be at best only a very rough approximation. As the acidity decreases, the values of R tend to approach a limit which we may take, for purposes of rough estimate, as 50, expressing the value in round numbers.

This makes possible an estimate, however hypothetical, of that increment of the conductivity which is due to protein chloride, or is

in any other way proportional to the quantity of combined acid. This value may then be subtracted from the corrected salt conductivity, thus giving as a final result that increment of the increase of conductivity which is not proportional to simple chemical action and which measures either the inaccuracies of the assumptions and approximations or the changes in the systems which are not due to phenomena already considered. The results of this calculation are given under X in Table II.

That part of the increase in conductivity which is proportional to protein salt formation may be supposed to result from the direct liberation of electrolytes by the union of the acid with the protein plus at least a moiety of the ionization of dissolved protein chloride.

The most obvious effects which have been neglected are the increasing rate of change of solubility of protein with increasing acidity and a corresponding change in the gluten mass, which involves the liberation of electrolytes. Both these factors ought to produce such a change in conductivity as is revealed by the data. For the protein which dissolves, whether chloride or not, will carry ions with it into the solution, and the disintegration of the gluten will favor the escape of electrolytes which might not otherwise find an open path into the aqueous phase.

It is therefore apparent that the simplest possible chemical phenomena, which are certainly all involved in the equilibria of the system, are quantitatively sufficient, within the range of accuracy of the measurements, to explain all the data. In short, the theoretical assumptions which are necessary upon purely chemical grounds are, within a very close approximation, also sufficient to account for the phenomena.

## III.

In order to proceed a step further in the investigation, it is necessary to study the relation between electrolytes in the solution and in the gluten phase. The following experiment bears upon this point. A series of systems, consisting of different quantities of powdered gluten and 100 cc. of distilled water of specific conductivity 9.6, was allowed to stand at a temperature of 22°C., and from time to time the conductivity was measured. The results are included in Table IV.

TABLE IV.

| Gluten in 100 cc. water. | Conductivity after | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 hr. | 2 hrs. | 3 hrs. | 4 hrs. | 5 hrs. | 6 hrs. | 27 hrs. |
| gm. | | | | | | | |
| 8 | | | 172. | | | 192. | 274. |
| 4 | | | 137. | | | 157. | 178. |
| 2 | 62.8 | 70.9 | 88.4 | 93.5 | 101. | 104. | 138. |
| 1 | | | 58.9 | | | 63.4 | 72.8 |
| 0.5 | | 35.7 | | | 42.6 | 40.7 | |
| 0.25 | 28.3 | 30.3 | 35.7 | 39.1 | 41.5 | 41.5 | |
| 0.125 | | 27.4 | | | 31.6 | 28.3 | |

Making allowance for the conductivity of the water, and reducing the measurements to the effect per gm. of protein, this experiment yields the results of Table V, which indicate that the amount of elec-

TABLE V.

| Gluten in 100 cc. water. | Relative solution of electrolytes per gm. of gluten after | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 hr. | 2 hrs. | 3 hrs. | 4 hrs. | 5 hrs. | 6 hrs. | 27 hrs. |
| gm. | | | | | | | |
| 8 | | | 20 | | | 23 | 33 |
| 4 | | | 32 | | | 37 | 42 |
| 2 | 27 | 31 | 39 | 42 | 46 | 47 | 64 |
| 1 | | | 49 | | | 54 | 63 |
| 0.5 | | 52 | | | 66 | 62 | |
| 0.25 | 75 | 83 | 104 | 118 | 128 | 128 | |
| 0.125 | | 142 | | | 176 | 150 | |

trolytes contained in 1 gm. of the gluten preparation is approximately sufficient to give a specific conductivity at 25°C. of 200 to 100 cc. of water. This rough estimate accords satisfactorily with determinations of the ash of the gluten:

| | *per cent* |
|---|---|
| I | 0.59 |
| II | 0.61 |
| Average | 0.60 |

These experiments illustrate the importance of the relation between quantity of protein and volume of solution in such systems, even when gluten is added to distilled water. When the variation of solubility and hydration of proteins under the influence of electrolytes are also

TABLE VI.

*Calculation of Conductivity of Ions Combined with 1 Gm. of Gluten at Different Hydrogen Ion Concentrations.*

| $R\left(\frac{HCl}{gluten}\right)$ | Weight of gluten. | | | |
|---|---|---|---|---|
| | 1 gm. | 2 gm. | 4 gm. | 8 gm. |
| 10 | 1,465 | | | |
| 5 | 1,123 | 1,191 | | |
| 2.5 | | 930 | 951 | |
| 2.0 | 775 | | | |
| 1.5 | | | 594 | |
| 1.0 | 483 | 498 | | |
| 0.5 | 346 | 341 | 339 | |
| 0.25 | | 235 | 236 | 255 |
| 0.125 | | | 197 | 214 |
| 0.0625 | | | | 166 |
| 0 | 155.5 | 157.7 | 164.8 | 165.5 |
| $R\left(\frac{NaOH}{gluten}\right)$ | | | | |
| 0.031 | | | | 172 |
| 0.062 | | | 171 | 188 |
| 0.125 | | 180 | 179 | 186 |
| 0.25 | 181 | 194 | 198 | 203 |
| 0.5 | 225 | 227 | 231 | 239 |
| 1.0 | 277 | 296 | 308 | |
| 2.0 | | 413 | | |
| 2.5 | | | 591 | |
| 4.0 | 509 | | | |

taken into account, it may be clearly seen how illusory are all conclusions which leave such factors out of consideration. At the same time, there is every indication in the regularity of the data that the final condition of equilibrium is a simple one. But this condition is slowly attained, and by a somewhat devious path.

The immediate conclusion to be drawn from these measurements is that the electrolytes originally present in the gluten are sufficient,

per gm. of gluten, to give a conductivity of approximately 200 to 100 cc. of water. This may now be used in further study of the data upon conductivity. For this quantity, plus the electrical conductivity of the solution of acid or alkali added to the gluten, minus the electrical conductivity of the resulting solution, may be taken as a roughly approximate measure of the quantity of electrolyte in union with protein. It is, however, a measure which cannot be directly employed for comparison of the acid systems with the alkaline systems, because of the considerable difference in conductivity of hydrochloric acid and sodiun hydroxide. And of course the various salts of the original gluten are also not equal in conductivity to the acid, to the alkali, or to each other.

The results of such calculations of the conductivity of electrolytes in union with gluten are collected in Table VI. It may easily be seen that the data vary in a regular and intelligible manner.

## IV.

In the light of the above facts the swelling of gluten in solutions of acid and alkalies may now be considered. The data of Table VII include observations upon the weight of the wet, swollen, coherent mass, upon the weight of dry gluten contained therein, as well as calculations of the amount of gluten dissolved and of the ratio of wet gluten to dry material contained within it (the true swelling).

Quantitatively the results are not at all points satisfactory. The following conclusions are possible. First, the weight of swollen gluten bears no relation to the true swelling (hydration) because of the marked variations in the quantity of protein dissolved. Second, it bears no direct relation to the hydrogen ion concentration, except when quantities of gluten and solution are kept constant. Third, the chief factor in determining the weight of swollen gluten is the quantity of protein which has been dissolved away from it. Fourth, the amount of gluten dissolved is greater the greater the acidity. Fifth, the true swelling of gluten is greater the greater the acidity. There also seems to be a tendency for the true swelling of gluten to increase as the relative quantity of gluten increases. This corresponds to a relatively greater amount of electrolyte present in the swollen mass.

TABLE VII.

*Measurements on Gluten (5.6 Gm.) in Equilibrium with Solutions of HCl and NaOH at Different Reactions.*

| pH | R | Gluten per 100 cc. solution. | | | |
|---|---|---|---|---|---|
| | | 1 gm. | 2 gm. | 4 gm. | 8 gm. |
| | $\left(\frac{HCl}{gluten}\right)$ | Weight of swollen gluten ($A$). | | | |
| | | gm. | gm. | gm. | gm. |
| 5.3 | 0.25 | | 11.5 | 11.1 | 13.1 |
| 5.5 | 0.125 | | | 12.9 | 14.3 |
| 5.6 | 0.0625 | | | | 14.5 |
| 5.7 | 0.0000 | 12.9 | 13.7 | 15.3 | 15.7 |
| | $\left(\frac{NaOH}{gluten}\right)$ | | | | |
| 5.9 | 0.0312 | | | | 15.8 |
| 6.0 | 0.0625 | | 14.2 | 15.4 | 16.0 |
| 6.3 | 0.125 | 14.2 | 15.1 | 15.7 | 15.6 |
| 6.8 | 0.25 | 15.1 | 15.1 | 15.2 | 15.4 |
| 7.5 | 0.50 | 14.6 | 14.8 | 15.0 | 15.3 |
| 9.4 | 1.00 | 13.6 | 13.9 | 14.3 | |
| | $\left(\frac{HCl}{gluten}\right)$ | Weight of dry gluten in 12 gm. of swollen gluten ($B$). | | | |
| 5.3 | 0.25 | | 3.75 | 3.74 | 3.22 |
| 5.5 | 0.125 | | | 3.90 | 3.89 |
| 5.7 | 0.0000 | | | | 4.08 |
| | $\left(\frac{NaOH}{gluten}\right)$ | | | | |
| 5.9 | 0.0312 | | | | 3.79 |
| 6.0 | 0.0625 | | 3.93 | 3.93 | 3.89 |
| 6.3 | 0.0125 | 4.04 | 3.93 | 3.93 | 4.01 |
| 6.8 | 0.25 | 3.99 | 4.05 | 4.08 | 4.00 |
| 7.5 | 0.50 | 4.16 | 4.18 | 4.05 | |
| 9.4 | 1.00 | | 4.12 | | |
| | $\left(\frac{HCl}{gluten}\right)$ | Amount of gluten dissolved $\left(5.6-\frac{A\cdot B}{12}\right)$. | | | |
| 5.3 | 0.25 | | 2.01 | 2.14 | 2.08 |
| 5.5 | 0.125 | | | 1.41 | 0.96 |
| 5.7 | 0.000 | | | | 0.67 |
| | $\left(\frac{NaOH}{gluten}\right)$ | | | | |
| 5.9 | 0.0312 | | | | 0.62 |
| 6.0 | 0.0625 | | 0.95 | 0.56 | 0.41 |
| 6.3 | 0.125 | 0.82 | 0.65 | 0.46 | 0.39 |
| 6.8 | 0.25 | 0.58 | 0.50 | 0.43 | 0.47 |
| 7.5 | 0.50 | 0.54 | 0.44 | 0.54 | |
| 9.4 | 1.00 | | 0.83 | | |

TABLE VII—*Concluded.*

| pH | R | Gluten per 100 cc. solution. | | | |
|---|---|---|---|---|---|
| | | 1 gm. | 2 gm. | 4 gm. | 8 gm. |
| | $\left(\frac{HCl}{gluten}\right)$ | Swelling of gluten $\left(\frac{12}{B}\right)$. | | | |
| | | gm. | gm. | gm. | gm. |
| 5.3 | 0.25 | | 3.20 | 3.20 | 3.72 |
| 5.5 | 0.125 | | | 3.08 | 3.08 |
| 5.7 | 0.000 | | | | 2.94 |
| | $\left(\frac{NaOH}{gluten}\right)$ | | | | |
| 5.9 | 0.0312 | | | | 3.17 |
| 6.0 | 0.0625 | | 3.05 | 3.05 | 3.08 |
| 6.3 | 0.125 | 2.97 | 3.05 | 3.05 | 2.99 |
| 6.8 | 0.25 | 3.01 | 2.96 | 2.94 | 3.00 |
| 7.5 | 0.50 | 2.88 | 2.87 | 2.96 | |
| 9.4 | 1.00 | | 2.92 | | |

## V.

Finally, we have measured the viscosity of swollen gluten with the help of the viscosimeter previously described. In every case 12 gm. of the swollen gluten were introduced into the apparatus.[3] The results of these measurements are included in Table VIII.

It should be understood that these measurements are of very moderate accuracy which, nevertheless, is sufficient for the present purpose. Evidently there is a well marked minimum of viscosity near a hydrogen ion concentration corresponding to pH 5.7. This minimum, as will be shown in a later paper, while due chiefly to the hydrogen ion concentration, is in a secondary manner influenced by the amount of electrolyte present in the system, and varies with this factor as well as with the acidity. The electrolyte concentration determines the great variation in viscosity measurements in the systems where the protein is suspended in distilled water. It will be shown in a later paper that the value of 3.0 for the system containing 8 gm. of gluten is most nearly comparable with the other data of the table.

[3] Henderson, L. J., Fenn, W. O., and Cohn, E. J., *J. Gen. Physiol.*, 1918–19, i, 387.

The measurements on smaller quantities of gluten in distilled water are accordingly disregarded. Averaging all the other values of Table VIII, which appear to be fairly comparable, the last column is obtained. This column represents more truly than the individual data the effect of variation of hydrogen ion concentration upon viscosity. It is graphically represented in Fig. 1.

The marked minimum in the viscosity of the gluten seems not to be dependent upon a single property. For it does not coincide with

TABLE VIII.

*Viscosity Measurements. Time in Minutes.*

| pH | R | Gluten per 100 cc. solution. | | | | |
|---|---|---|---|---|---|---|
| | | 1 gm. | 2 gm. | 4 gm. | 8 gm. | Average. |
| | $\left(\frac{HCl}{gluten}\right)$ | Weight, 350 gm. | | | | |
| 5.3 | 0.250 | | | | 16.0 | 16.0 |
| 5.5 | 0.125 | | | 10.8 | 7.5 | 9.2 |
| 5.6 | 0.062 | | | | 5.8 | 5.8 |
| 5.7 | 0.000 | 55.0 | 28.0 | 5.5 | 3.0 | 3.0 |
| | $\left(\frac{NaOH}{gluten}\right)$ | Weight, 450 gm. | | | | |
| 5.9 | 0.0312 | | | | 2.8 | 2.8 |
| 6.0 | 0.0625 | | 5.8 | 5.0 | | 5.4 |
| 6.3 | 0.125 | 6.3 | 6.8 | 4.0 | 5.8 | 5.7 |
| 6.8 | 0.25 | 6.3 | 5.0 | 7.0 | 6.3 | 6.2 |
| 7.5 | 0.50 | 12.5 | 9.8 | 10.3 | 15.3 | 12.0 |
| 9.4 | 1.0 | 18.0 | 17.5 | 13.5 | | 16.3 |

the isoelectric point—according to Wood and Hardy[4] probably between $10^{-7}$ N and $10^{-8}$ N—and it does not correspond with the true swelling, or hydration. It is, moreover, as the observations in the presence of distilled water show, greatly influenced by the concentration of electrolytes. This last point will be fully established in a later paper. Finally, it appears to be largely variable with time, so that a process of "setting" may be suspected.

[4] Wood, T. B., and Hardy, W. B., *Proc. Roy. Soc. London, Series B*, 1909, lxxxi, 39.

In short, the viscosity of gluten seems to depend upon the hydrogen ion concentration, upon the amount of water and of electrolytes present in the swollen mass, and at times upon the age of the system. These are, however, the factors whose importance was to have been expected. A more complete interpretation of their mode of action is at present impossible. But the well marked minimum of viscosity in an acid range of reaction is plain, and, as we hope to show, of decisive practical importance in bread making.

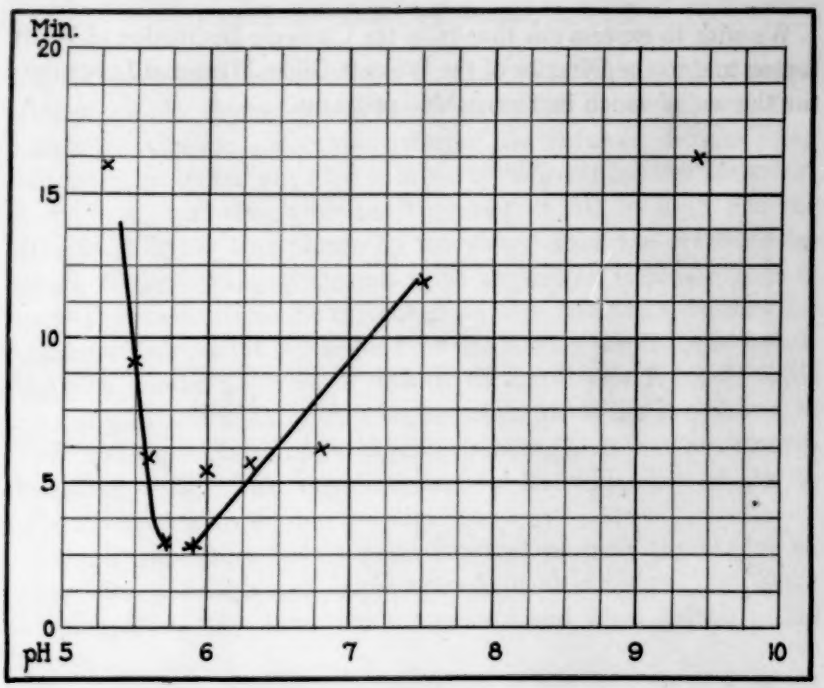


FIG. 1.

## SUMMARY.

In this paper there are reported studies of the acid-base equilibrium in systems containing gluten suspended in solution of hydrochloric acid and sodium hydroxide. The studies have involved measurements of the hydrogen ion concentration, of the electrical conductivity, and of the solution of the proteins. Further, measurements have

been made of the swelling and of the viscosity of the gluten component of such systems.

The results seem to show that simple chemical phenomena are most important in such systems, and that the modifications of these, resulting from colloidal and heterogeneous characteristics, are of secondary importance in determining the condition of equilibrium, though somewhat more significant in the progress of the system toward the condition of equilibrium.

We wish to express our thanks to the Carnegie Institution of Washington and to the Director of the Wolcott Gibbs Memorial Laboratory for the use of much indispensable apparatus.

# RELATION BETWEEN THYROID GLAND, METAMORPHOSIS, AND GROWTH.

By EDUARD UHLENHUTH.

*(From the Laboratories of The Rockefeller Institute for Medical Research.)*

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It has been demonstrated by a number of workers that the active principle of the thyroid gland in the causation of amphibian metamorphosis is iodine. Recently Swingle[1] has obtained definite proof that inorganic iodine when fed to larvæ of *Salientia* induces metamorphosis in a short time after the beginning of the feeding. On the other hand, it has been shown by the writer[2] that the retarding influence of the feeding of thymus upon amphibian metamorphosis is due to the absence from the thymus gland of a substance required for metamorphosis, and it is possible that this lacking substance is iodine. Although minute amounts of iodothyrin have been found in some thymus glands, it is likely that the amounts found are insufficient to produce metamorphosis and that some thymus glands may not contain the iodine at all. This would account for the variability of the results obtained with thymus feeding.

Swingle[1] also subjected tadpoles deprived of their thyroid to an iodine diet; although such larvæ when kept on a normal diet never metamorphose, they very soon metamorphosed when fed on iodine crystals.

Hence it is manifest that iodine is one of the substances involved in the causation of amphibian metamorphosis. The quantities contained in normal food are, however, so small as to have no immediate effect upon the organism. If the larvæ have no thyroid glands, the small quantities of iodine taken up with the food apparently cannot be retained by the organism and the iodine leaves the body without

[1] Swingle, W. W., *J. Exp. Zool.*, 1919, xxvii, 397.

[2] Uhlenhuth, E., *J. Gen. Physiol.*, 1918–19, i, 305.

bringing about metamorphosis. If, however, a thyroid gland is present, all or most iodine taken up by the organism is retained and stored up in the thyroid. At a certain time during the life of the larva the thyroid suddenly begins to excrete the iodine stored up during the larval period, and metamorphosis results.

From this it is evident that under normal conditions the iodine is not the only substance needed in metamorphosis; there must be still another substance which, when present in a certain quantity, causes the thyroid to excrete the thyroid hormone. The experiments to be reported in this article not only furnish evidence of the actual existence of such an excretor substance, but they also demonstrate that it is evolved during the processes which lead to the growth of the organism.

### *Metamorphosis and Rate of Growth in Worm-Fed Larvæ.*

In eleven series of larvæ of the species *Ambystoma opacum* the rate of growth during the larval period of active growth has been determined in the following way.

In each series the time of metamorphosis was recorded for each single individual; this can be done very accurately in this species if the first shedding of the skin and the reduction of the gills to mere stumps without fringes are taken as the indication of metamorphosis, both phenomena occurring within a day. That these two processes are actually controlled by the influence of the thyroid, in contradistinction to many other processes, has been indicated already in former articles[3] and will be shown in detail in a later publication. From the values obtained in this way the average length of time of the larval period was calculated for each series (Table I).

Each individual was measured once a week and the average sizes obtained from those values for each series were plotted in curves. At the time of metamorphosis a sharp drop of the curve takes place, due to a discontinuation of growth, and even a diminution of the size of the animals which may last for one or several weeks before growth is resumed. In Series A 1916, the curve of which is shown in Fig. 1,

[3] Uhlenhuth, E., *J. Exp. Zool.*, 1917–18, xxiv, 237; 1918, xxv, 135; *J. Gen. Physiol.*, 1918–19, i, 305.

this drop occurred in the 24th week, though less distinctly than in most of the other series. From the average sizes reached in each series when the drop occurred and from the time when the drop took place, the rate of growth was calculated in day-millimeters, as indicated in Column 3 of Table I.

In comparing the length of the larval period for each series with the corresponding rate of growth it becomes evident that the smaller the rate of growth observed, the later metamorphosis took place. Furthermore, the rates of growth appear to be proportional to the

TABLE I.

*Rate of Growth (R) in Worm-Fed Ambystoma opacum during Larval Period of Active Growth (Calculated from Averages).*

| Series. | Age at metamorphosis. | R | R × A |
|---|---|---|---|
| | *days* | | |
| C 1916 | 245 | 0.27 | 66 |
| A 1916 | 182 | 0.31 | 56 |
| E 1917 | 161 | 0.36 | 58 |
| D 1917 | 127 | 0.48 | 61 |
| $W_K$ 1917 | 106 | 0.58 | 62 |
| $W_{Na}$ 1917 | 100 | 0.58 | 58 |
| $W_{Mg}$ 1917 | 100 | 0.58 | 58 |
| W 1917 | 97 | 0.62 | 60 |
| $W_{Ca}$ 1917 | 96 | 0.62 | 60 |
| C 1917 | 80 | 0.79 | 63 |
| XIV 1918 | 70 | 0.83 | 58 |
| Average | | | 60 |

velocity of metamorphosis; for the product of the rate of growth into the duration of the larval period (R × A) is constant, as may be seen from Table I, the average value of R × A being 60.

This means that during the process of growth a substance is evolved which when present in a definite amount induces metamorphosis, provided that the larvæ have been fed on normal food which apparently contains enough iodine to furnish the other substance (iodine) required for metamorphosis in a sufficient quantity; the greater the rate of growth the quicker that quantity of the first substance is formed which is required to induce the secretory action of

the thyroid gland. Hence besides iodine still another substance is needed in the amphibian metamorphosis; namely, the excretor substance which causes the thyroid to excrete the stored up iodine.

It may be mentioned here that the sudden drop of the growth curve at the time of metamorphosis may be explained if we assume that the thyroid, when it is stimulated by the excretor substance, excretes at

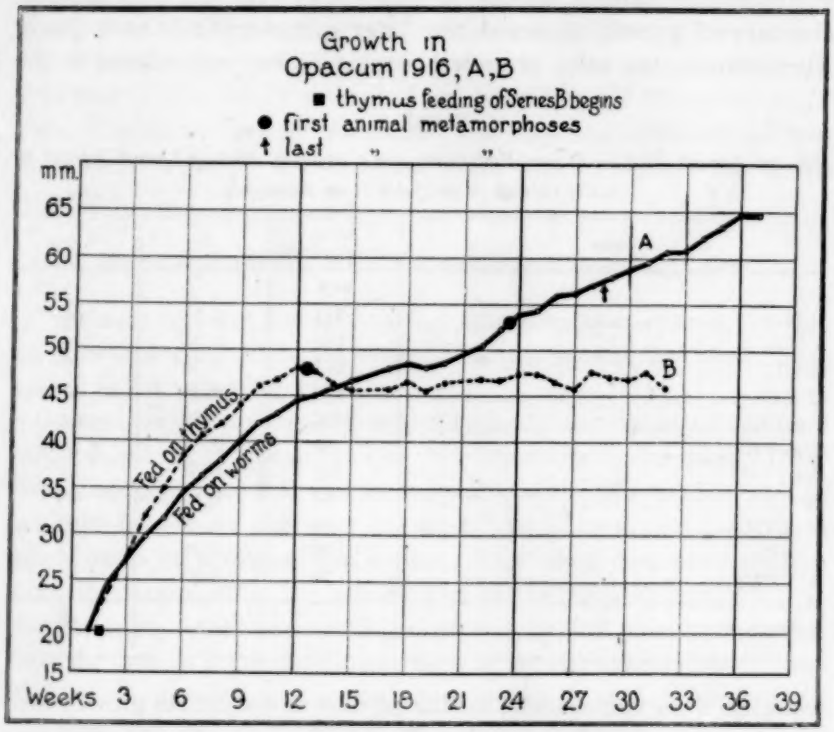



FIG. 1.

first an overdose of iodine, while later the excretion becomes less energetic. Janney[4] has shown that while an overdose of thyroid hormone leads to a negative nitrogen balance and a loss in weight and size, a certain minimum dose results in a positive nitrogen balance and a gain in weight and size.

[4] Janney, N. W., *Arch. Int. Med.*, 1918, xxii, 187.

*Metamorphosis and Rate of Growth in Thymus-Fed Larvæ.*

In a recent publication Janney[4] has shown that while a certain minimum amount of the thyroid hormone results in an increased protein breakdown, it finally leads to a positive nitrogen balance and an increase in weight and size, since it not only accelerates the protein breakdown but at the same time facilitates the assimilation of the nitrogen into the proteins of the body tissues. This is shown also in the metamorphosis of the amphibians where the initial breakdown of the tissues, as demonstrated, for instance, by Morse[5] on tadpoles, and the decrease in size, resulting probably, as suggested above, from the excretion of an overdose in the beginning of the functional period of the thyroid, are followed soon by an increase in size and weight.

On the other hand, Janney's experiments have shown that in certain diseases, such as exophthalmic goiter, the normal synthesis by the thyroid of the thyroid hormone from iodine and certain organic substances is disturbed, and on the basis of Swingle's experiments we may assume that in these diseases the thyroid is unable to retain the iodine consumed by the organism with the food. This assumption is supported by the fact, as mentioned by Janney, that in exophthalmic goiter the thyroid frequently is found very poor in iodine. But since in this case the excretory function of the thyroid remains undisturbed, the thyroid is excreting, instead of the hormone, certain substances, probably the indole-containing amino-acid tryptophane which normally is used to build up the hormone, but which in itself is toxic causing a permanent protein breakdown without facilitating assimilation of the food nitrogen; consequently a negative nitrogen balance is brought about and a permanent loss of weight.

If we apply this hypothesis to amphibian metamorphosis, we should expect that in such larvæ, which have not been able to take up an amount of iodine sufficient for metamorphosis and whose thyroid consequently was unable to develop the normal hormone, the action of the excretor substance would lead to a prolonged and increased loss in size of the larvæ, without resulting metamorphosis, since in these animals excretion by the thyroid would commence as soon as the excretor substance reached the amount required, but the excreted

[5] Morse, W., *Biol. Bull.*, 1918, xxxiv, 149.

substance would be the toxic substance tryptophane instead of the normal hormone.

This expectation is actually fulfilled in the thymus-fed larvæ of *Ambystoma opacum*, as is shown by Curve B, Fig. 1. In this curve is represented the growth of a series of larvæ of the same age and from the same mother as the larvæ used for the experiment plotted in Curve A; both series were kept under the same conditions, but while Series A was fed on earthworms, Series B was fed on thymus. The thymus-fed larvæ grew normally in the beginning—and in this particular series even more quickly than the controls, a fact explained by the writer in a previous paper.[6] At the 13th week we observe a sharp drop in Curve B and hereafter growth was stopped and never resumed again. Exactly the same results were obtained for the thymus-fed larvæ of *Ambystoma maculatum* and *Ambystoma tigrinum*.

The time when the drop of the curve takes place in the thymus-fed animals is that at which metamorphosis should have occurred if they had received normal food. This was not only suggested by the general appearance of these larvæ and by the fact that a few larvæ actually did metamorphose (in Series B 1916 only one larva metamorphosed at this time), but it can be proved if we calculate the time of metamorphosis from the value of the product R × A for the worm-fed larvæ. This has been done in Table II for eight thymus-fed series of *Ambystoma opacum*. Again the product R × A was calculated from the duration of the larval period and the rate of growth, the latter value being obtained as in the worm-fed larvæ from the growth curve up to the point where the drop occurred. First we notice that the product R × A (Table II) in the first three series is far above the average value of R × A as obtained for the worm-fed series. This is due to the fact that the larvæ of these series did not metamorphose at a time proportional to their rate of growth but much later, due to the absence of iodine at this time. The time at which they should have metamorphosed if iodine had been present in the food in a normal amount, can be calculated, however, from the product R × A in the worm-fed series, which is 60, and the rate of

[6] Uhlenhuth, E., *Proc. Soc. Exp. Biol. and Med.*, 1917–18, xv, 37.

growth in each particular thymus-fed series. We find, then, that in the first three series metamorphosis was greatly retarded (Column 5, Table II). In Series D 1916 metamorphosis should have taken place at 162 days instead of at 207 days, in Series B 1916 at 109 days instead of at 133 days, and in Series $T_{Ca}$ 1917 at 113 days instead of at 122 days. In the other series the retardation of metamorphosis, if there was any at all, was only slight; how far this was due to the special treatment these thymus-fed animals received will not be discussed here. It may be mentioned, however, that in the last series (T 1917) the retardation of metamorphosis was prevented probably

TABLE II.

*Rate of Growth (R) in Thymus-Fed Ambystoma opacum during Larval Period of Active Growth (Calculated from Averages).*

| Series. | Age at metamorphosis. | R | R × A | Age at which animal should metamorphose. |
|---|---|---|---|---|
| | *days* | | | *days* |
| D 1916 | 207 | 0.37 | 77 | 162 |
| B 1916 | 133 | 0.55 | 73 | 109 |
| $T_{Ca}$ 1917 | 122 | 0.53 | 65 | 113 |
| B 1917 | 122 | 0.50 | 61 | 120 |
| $T_{Na}$ 1917 | 122 | 0.50 | 61 | 120 |
| $T_{Mg}$ 1917 | 109 | 0.58 | 63 | 103 |
| $T_{K}$ 1917 | 104 | 0.57 | 59 | 105 |
| T 1917 | 101 | 0.60 | 61 | 100 |
| Average | | | 65 | |

by adding parathyroid to the thymus diet; since the parathyroids are known to contain small amounts of iodine, it is probable that the thyroid of these larvæ was apparently able to store up enough iodine to permit normal thyroid excretion and metamorphosis when the excretor substance began to act.

The number of days at which the second thymus-fed series (B 1916) should have metamorphosed, *i.e.* at which the excretor substance began to act, is 109. This approaches very closely the time when the drop of the curve occurred (95 days), if we consider that the value 109 was calculated only from an average value of R × A.

If prevention of metamorphosis by the absence of iodine, but in the presence of a thyroid gland, is accompanied by a check in growth (for the reasons given above), we should expect that prevention of metamorphosis in the absence of a thyroid gland would not be accompanied by an abnormal check of growth. Hence amphibian larvæ deprived of their thyroids should be able to complete the normal growth of the species without any disturbances. That this is actually the case is shown in Allen's tadpoles which were deprived artificially of their thyroid glands and which reached frequently enormous sizes, becoming real giant larvæ. A similar phenomenon is found in such forms as *Typhlomolge rathbuni*, which for some reason do not develop a thyroid gland—as discovered by Emerson[7]—and hence remain permanently in a larval condition, but which seem able to grow for years without disturbances.

Thus the experiments reported in this article seem to prove that metamorphosis of the normal larvæ of *Ambystoma opacum* which possess a thyroid depends not only upon the presence of a sufficient amount of iodine in this gland but also on the presence of the action of a second substance inducing the excretion of the iodine by the thyroid gland. By means of this hypothesis we are able to explain why thymus-fed larvæ suddenly stop growing at the time when metamorphosis should occur, without, however, metamorphosing, and why species not possessing thyroid glands, such as the *Typhlomolge*, can complete their growth without disturbance.

### *Metamorphosis and Temperature.*

There is still another phenomenon which lends itself readily to explanation on the basis of the assumption of an excretor substance. It is a well known fact that growth is retarded at low temperature, and since we have seen that the excretor substance is evolved during growth, it is not surprising that metamorphosis also should be retarded at low temperature. This has long been observed by many students of amphibian metamorphosis. But what remains unexplained is the fact that amphibian larvæ when kept in low temperature are always much larger at the time of metamorphosis than

[7] Emerson, E. T., *Proc. Boston Soc. Nat. Hist.*, 1905, xxxii, 43.

they would be if kept at high temperature. We have observed this phenomenon frequently and in looking for an explanation have tried to find a relation between the size of the larvæ and the age at the time of metamorphosis, similar to that existing between the latter quantity and the rate of growth. Such a relation, however, does not exist.

But if we assume the action of an excretor substance in metamorphosis, the phenomenon in question can be readily explained. Comparing R × A for the first worm-fed series (C 1916) in Table I, which was kept at a temperature of 10°C. below that of the other series, with the rest of the series of Table I, we observe that it is very high (10 per cent) above the average, which would indicate that metamorphosis in this series was more retarded than the corresponding rate of growth would demand. Since in this series a drop of the growth curve similar to the drop of the growth curve of the thymus-fed animals did not occur, this case of undue retardation of metamorphosis cannot be explained in the same way as in the thymus-fed larvæ, *i.e.* by asuming that from a lack of iodine in the thyroid gland a destructive compound was excreted by the thyroid. The only way to explain this case of retardation is to assume that at low temperature less excretor substance is evolved than at high temperature during an equal rate of growth processes. That this should be possible is not at all surprising, but was to be expected since it is well known that the temperature coefficients for different physiological processes may differ greatly. Loeb, for instance, pointed out that not only the temperature coefficients but also their variations at the lower and upper temperature scale differ considerably in different physiological processes.[8]

But if the amount of excretor substance produced by an equal rate of growth is less at low temperature than at high temperature, the animals kept at low temperature must grow longer at an equal rate than those kept at high temperature before that amount of excretor substance is produced which is required to bring about thyroid excretion. Consequently the low temperature larvæ must reach a larger size than the high temperature larvæ, before they can metamorphose.

[8] Loeb, J., Mechanistic conception of life, Chicago, 1912, 212.

The same phenomenon was observed also in the thymus-fed larvæ (Series D 1916, Table II) and since here the lack of iodine and that of the excretor substance are combined, it is not surprising that the value for R × A is still higher (28 per cent) above the average than in the low temperature series of the worm-fed larvæ (Series C 1916, Table I).

Thus with the hypothesis of an excretor substance we can explain a phenomenon which for a long time was confusing to experimental biologists as well as to systematists.

## SUMMARY.

1. Two substances are involved in amphibian metamorphosis as studied in *Ambystoma opacum:* first, iodine, which is taken up by the food, and second, an excretor substance, which is evolved during the processes of growth and serves to induce the excretory function of the thyroid gland.

2. This explains why in larvæ, whose metamorphosis is inhibited by lack of iodine, growth is checked at the time when metamorphosis should occur; for at this time the excretor substance commences to act and this results, if iodine is absent, in the excretion by the thyroid of toxic substances which cause the breakdown of proteins and consequently a decrease in size of the larvæ.

3. Larvæ whose metamorphosis is inhibited by extirpation of the thyroid or by the hereditary lack of a thyroid (as is the case in *Typhlomolge*) can grow normally, since in them the action of the excretor substance cannot result in the excretion by the thyroid of a toxic growth-inhibiting substance.

4. At low temperature less excretor substance is produced than at high temperature during an equal rate of growth; therefore larvæ kept at low temperature reach a larger size than larvæ kept at high temperature, before they metamorphose.

The writer wishes to express his thanks to the Library of the Brooklyn Museum, and especially to Miss S. H. Hutchinson, for courtesies extended to him.

# AMPHOTERIC COLLOIDS.

## IV. The Influence of the Valency of Cations upon the Physical Properties of Gelatin.

BY JACQUES LOEB.

*(From the Laboratories of The Rockefeller Institute for Medical Research.)*

(Received for publication, January 22, 1919.)

### I. INTRODUCTION.

In 1901 and 1902 the writer[1] published a series of investigations in which he showed that low concentrations of bivalent cations, practically without regard to their chemical nature, *e.g.* Mg, Ca, Sr, Ba, Zn, Co, Pb, etc., inhibited the toxic action of high concentrations of salts with univalent cations, upon the eggs of *Fundulus*. Trivalent cations like $AlCl_3$ and $CrCl_3$ seemed to act still more effectively than the bivalent ions though it was obvious that secondary influences (*e.g.* the high hydrogen ion concentration) restricted the limit of their antagonistic influence. This influence of valency the writer attributed to the effect of the electric charges of the ions upon the physical state of the colloidal material. He also found that polyvalent anions, *e.g.* $SO_4$, oxalate, citrate, etc., had under the same conditions no antagonistic effect. Since that time cases of an antagonistic action of polyvalent anions have come to light, but this phenomenon is not only less common but also less striking than the antagonistic effect of polyvalent cations.

It had been shown in the writer's preceding publications on gelatin[2] that the salts of gelatin with univalent cations possess a comparatively high osmotic pressure, a high viscosity, a high degree of swell-

[1] Loeb, J., *Arch. ges. Physiol.*, 1901–02, lxxxviii, 68; *Am. J. Physiol.*, 1901–02, vi, 411.

[2] Loeb, J., *J. Biol. Chem.*, 1918, xxxiii, 531; xxxiv, 77, 395, 489; xxxv, 497; *J. Gen. Physiol.*, 1918–19, i, 39, 237, 363.

ing, and a high alcohol number, while the gelatin salts with bivalent cations have a much lower osmotic pressure, lower viscosity, etc. It was also found that the addition of a certain amount of a salt with a bivalent or polyvalent cation depresses the effect of salts with monovalent cation on osmotic pressure, viscosity, swelling, etc., of the gelatin.

Pauli[3] and Michaelis[4] seem to ascribe the variations in the swelling and the viscosity of protein solutions to variations in the degree of ionization and to a "hydratation" they assume to be connected with the ionization of the protein. According to this view we should have to assume that sodium gelatinate has a higher osmotic pressure than calcium gelatinate of the same concentration, because the former is more strongly ionized. In an earlier paper the writer tentatively accepted Pauli's hypothesis, but a closer scrutiny of the literature showed that neither Pauli nor Michaelis measured the effect of electrolytes upon the conductivity of their protein solutions, probably on account of the fact that they did not remove the excess of electrolyte after it had acted on the protein. The writer's method of removing the excess of electrolytes after they have had time to react with the gelatin made measurements of conductivity possible, and these measurements in connection with measurements of osmotic pressure and of the quantity of metal in combination with the gelatin led to a very definite explanation of the influence of the valency of ions on the properties of gelatin. *With the same equivalent of metal in combination with a given mass of gelatin the maximal osmotic pressure of a 1 per cent solution of gelatin salts with univalent cation, e.g. Na gelatinate, is almost exactly three times as great as that of gelatin salts with a bivalent metal, e.g. Ca gelatinate, while the conductivities of the solutions of the two types of gelatin differ little or not at all.* This indicates that the gelatin salts with univalent metal have at the point of maximal osmotic pressure about three times as many particles in solution as the same mass of gelatin salts with bivalent metal, while the number of electrical charges is about the same in both cases. The identity of the conductivities of gelatin salts of the type of sodium gelatinate and calcium

[3] Pauli, W., *Fortschr. naturwiss. Forschung*, 1912, iv, 245.
[4] Michaelis, L., Die Wasserstoffionenkonzentration, Berlin, 1914.

gelatinate proves that the difference in the influence of the valency of the cation upon the physical properties of gelatin cannot be ascribed to a difference in the degree of ionization of the two types of salts, but is due to some other as yet unknown factor. It seems possible to explain all the phenomena on the basis of the tentative assumption that gelatin salts with a bivalent cation dissociate into cations and aggregates of four, or six, or eight gelatin ions, each individual gelatin ion of the aggregate retaining its original negative charge, while the anions of salts of sodium gelatinate consist of only one gelatin ion each. The quantitative data suggest that the number of gelatin anions contained in each aggregate is of a stoichiometrical order, being a simple multiple of the number representing the valency of the polyvalent metal ion.

### *II. Amount of Alkali Combining with Gelatin.*

We used powdered Cooper's non-bleached gelatin which is impure, having a pH of about 7.0 and consisting to a large extent of calcium gelatinate. It is necessary to purify this gelatin before using it by bringing it to the isoelectric point pH = 4.7.[5] This is done by putting 1 gm. of gelatin for ½ hour into 100 cc. of 3 M/1024 HCl or M/128 acetic acid, then putting the gelatin on a filter, allowing the excess of solution to drain off, and washing the gelatin two or three times with 25 cc. of distilled water at 5°C. If we wish to transform the pure isoelectric gelatin into a metal gelatinate we treat it subsequently with the hydroxide of the metal—*e.g.* NaOH, $Ca(OH)_2$, etc.—with which we wish the gelatin to combine.

We always used finely powdered gelatin rendered isoelectric in the manner described. When we intended to prepare sodium gelatinate we treated different doses of isoelectric gelatin of 1 gm. each with 75 or 100 cc. of a NaOH solution varying from M/4 to M/8192 NaOH, after-

[5] Isoelectric gelatin does not react with neutral salts like NaCl but will react with NaOH, since in this latter case the pH is raised beyond that of the isoelectric point. Common Cooper's gelatin, with a pH = 7.0, consisting of gelatin salts, especially Ca gelatinate, will be influenced in the same way by a treatment with NaOH as with NaCl, since in both cases a replacement of Ca by Na will occur. This is supported by the writer's previously published papers.

wards washing away the excess of alkali. In each case sodium gelatinate was formed but the amount formed varied with the concentration of the alkali solution used; and the pH varied correspondingly. It was of importance to measure the amount of Na or Ca in combination with the gelatin, to make sure that we were dealing with phenomena of a stoichiometrical character; it was especially necessary to make sure whether or not Na and Ca combine with gelatin in equivalent proportions. The metal gelatinate used for this purpose was not only washed as indicated, but was also dialyzed over night through collodion bags against 400 cc. of distilled water.

Our former experiments[6] allow us to measure the amount of metal contained in a given mass of gelatin when the pH is known. Metal gelatinates can only exist on the alkaline side from the isoelectric point of gelatin, this point being defined by a pH = 4.7. The Na or K or Ca in combination with the gelatin at each pH can be calculated in the following way. We determine the cc. of 0.01 N NaOH required to bring 25 cc. of 1 per cent isoelectric gelatin of different pH (lying between 4.7 and 7.0) to the point of neutrality (pH = 7.0). By deducting this value from the quantity required to bring 25 cc. of 1 per cent gelatin solution from the isoelectric point to pH = 7.0—which was always found to be about 4.5 cc. of 0.01 N NaOH[7]—we obtain the amount of 0.01 N Na or of ½ Ba or ½ Ca in combination with the 25 cc. of 1 per cent gelatin at any pH between 4.7 and 7.0. When pH is > 7.0 we ascertain the amount of 0.01 N HCl required to bring the 25 cc. of gelatin to pH = 7.0, and add this to the value 4.5. In Fig. 1 the abscissæ are the pH, the ordinates the cc. of 0.01 N Na, K, or ½ Ba found in combination with 25 cc. of gelatin for each pH. The results of three different experiments with the three different alkalies named are plotted (Fig. 1), showing the degree of agreement of the results. The curves are exactly the same whether gelatin has been treated with KOH, NaOH, LiOH, $Ba(OH)_2$, or $Ca(OH)_2$. It is also obvious from the curves that we are dealing with a simple salt formation of

[6] Loeb, J., *J. Gen. Physiol.*, 1918–19, i, 363.

[7] In our preceding paper we determined the cc. of 0.01 N NaOH required to bring 25 cc. of 1 per cent gelatin from the isoelectric point to the turning point of phenolphthalein (pH = 9.0) and found 5.5, which agrees with our present result where we neutralize to pH = 7.0 instead of to 9.0.

a stoichiometrical nature and that one Ba or Ca replaces two Na (or two Li, K, or $NH_4$). The maximum amount of salt formed is practically reached not far beyond pH = 8.0, which is so near the point of neutrality that practically no corrections for the values for titration and only slight corrections for the values for conductivity are required. Our conductivity curves are the corrected curves, *i.e.* from the measured values the conductivities of the pure alkali solutions of the same pH are deducted.

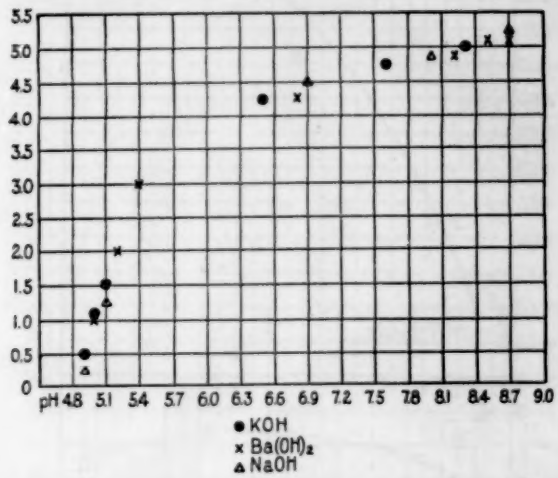
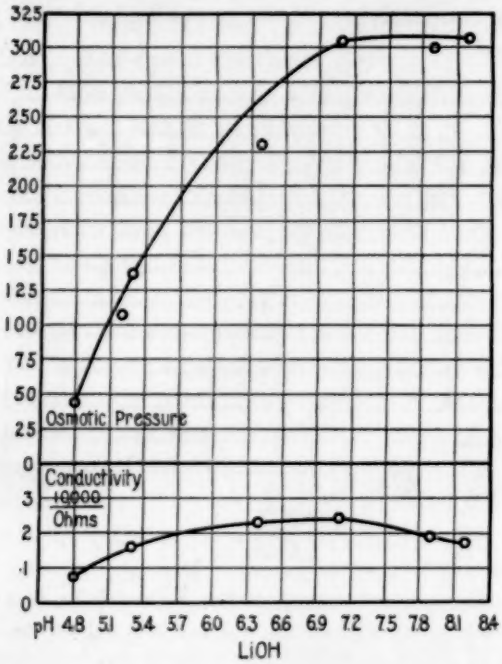



FIG. 1. Ordinates represent amount of Na, K, and ½ Ba (expressed in cc. of 0.01 N Na) in combination with 25 cc. of 1 per cent gelatin solution, previously treated with NaOH, KOH, or $Ba(OH)_2$ respectively and freed from the excess of salt by washing. Abscissæ represent pH of the solution. Curves in all three cases are identical, showing that one Ba replaces two Na or K.

## *III. Action of Different Alkalies on the Conductivity and Osmotic Pressure of Gelatin.*

In order to obtain constant results we brought the powdered gelatin, as stated, to the isoelectric point by treating it for 30 minutes with M/128 acetic acid at 20°C. The gelatin was then put on a filter, the acid allowed to drain off, and then it was washed twice with 25 cc. of distilled water at about 5°C. Then such gelatin, while on the filter, was perfused three times with 25 cc. of a solution of NaOH or KOH,

etc., of a definite concentration, varying from M/4 to M/8192 at 15°C. During this perfusion the powdered gelatin was sufficiently stirred to allow intimate contact between all the gelatin particles and the alkali, and then after all the excess of alkali had been allowed to drain off the mass on the filter was washed once with 25 cc. of $H_2O$ at 5° and




FIGS. 2 to 7. Curves for osmotic pressure (in terms of mm. of a 1 per cent gelatin solution) and conductivity $\left(\frac{10{,}000}{\text{ohms}}\right)$ of a 1 per cent gelatin solution first rendered isoelectric and then treated with concentrations of an alkali, *e.g.* NaOH, varying from M/8 to M/8192 to cause a varying proportion of the gelatin to form a metal gelatinate. Abscissæ represent pH of the solution after dialysis; ordinates of upper curve, osmotic pressure, and of lower curve, conductivity of the solution found at different pH. Curves for the osmotic pressures for gelatin salts with univalent ion, Li, Na, K, and $NH_4$ (Figs. 2 to 5) are alike and high, reaching a maximum of about 325 mm. Curves for the osmotic pressures of salts with bivalent metals, Ca and Ba, are very much lower than those for metal gelatinates with univalent metal, reaching a maximum of only 125 mm. Curves for conductivity are almost identical for both types of gelatin salts.

once with 25 cc. of $H_2O$ at 20°C. Then the mass was made into a 1 per cent solution and put into collodion bags to determine the osmotic pressure. Each collodion bag was surrounded by a beaker containing 400 cc. of distilled water and the temperature was kept constant at 24°C. This allowed any excess of alkali left to diffuse out of the bag. The next day the osmotic pressure was measured, the pH of the gela-

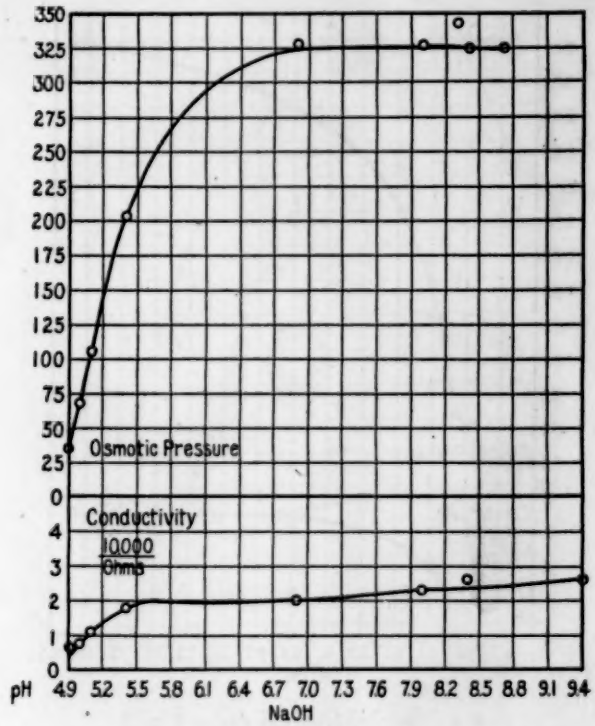



FIG. 3. See explanation under Fig. 2.

tin solution was determined, the solution titrated to determine the quantity of Na or K, etc., in combination with the gelatin, and the conductivity of the solution was measured.

The results of these measurements are contained in Figs. 2 to 7. The abscissæ are the pH, the ordinates the values for conductivity and osmotic pressure for these pH. The reader will notice that the curves representing the influence of Li, Na, K, and $NH_4$ on the osmotic

pressure (and the other physical properties) of gelatin are identical, if we use the pH as abscissæ. This result contradicts the statements current in colloid chemistry according to which these four cations have a different effect. The colloid chemists who make such statements have failed to measure the hydrogen ion concentration of their solutions. Our experiments show that the effects of the same cation on

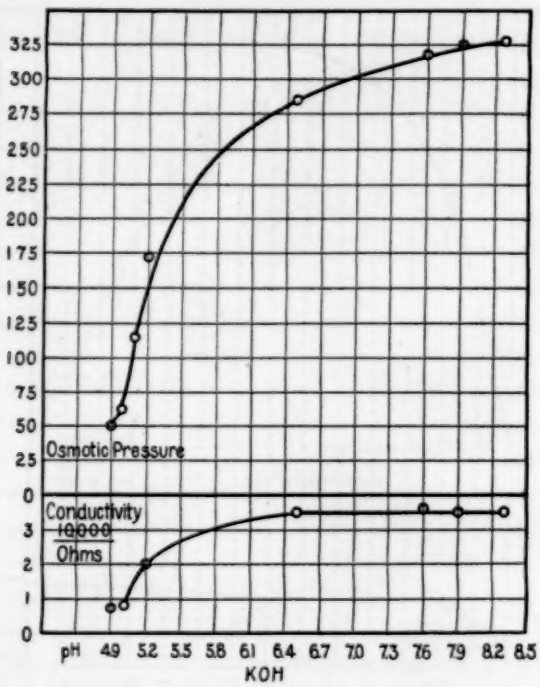



FIG. 4. See explanation under Fig. 2.

gelatin differ for different pH and hence we cannot be sure that apparent differences in the effect of two cations on a protein are the expression of differences in the structure of the two cations, unless we are certain that the pH is the same in both cases. The colloid chemists have, moreover, compared the effects of neutral salts of Li, Na, K, and $NH_4$, in the presence of an excess of these salts, which introduces a second error.

It is obvious that the osmotic pressure in the curves for LiOH, NaOH, KOH, and $NH_4OH$ (Figs. 2 to 5) reaches the same maximum (at pH between 7.0 and 8.0), namely that of a column of about 325 mm. of a 1 per cent gelatin solution; and that the curves for osmotic pressure of the Ca gelatinate and Ba gelatinate (Figs. 6 and 7) reach also an equally definite maximum of about 125 mm. osmotic pressure at about the same pH. In both cases we have probably to deduct

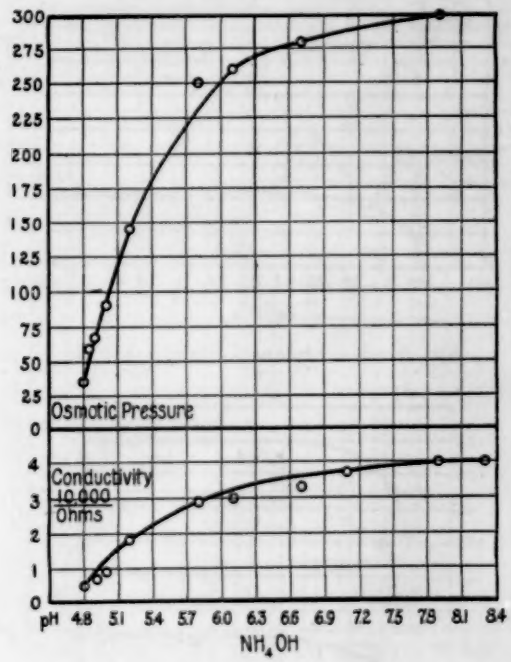



FIG. 5. See explanation under Fig. 2.

from these values about 25 mm.; namely, the osmotic pressure of a 1 per cent gelatin solution at the isoelectric point which includes the necessary capillary correction. This then leaves the following characteristic and constant values for the maximum osmotic pressure of the two types of metal gelatinates:

Li, Na, K, $NH_4$ gelatinate.....300 mm. (uncorrected value 325 mm.)
Ca and Ba gelatinate.....100 " ( " " 125 " )

The constant character of this ratio of the two pressures of 1 : 3 (or 3 : 8 for the uncorrected value) for the two valencies, but regardless of the other qualities of the ions, betrays a stoichiometrical basis for the influence of valency. The experiments were repeated to guard against error, the results remaining the same.

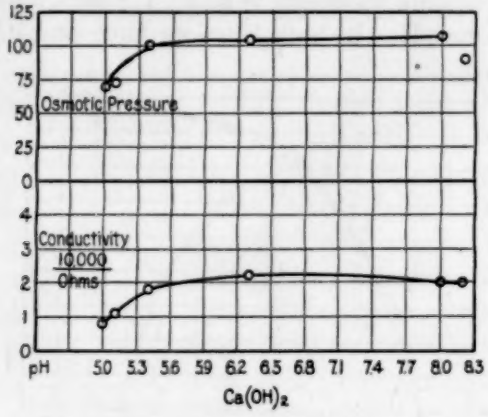



FIG. 6. See explanation under Fig. 2.

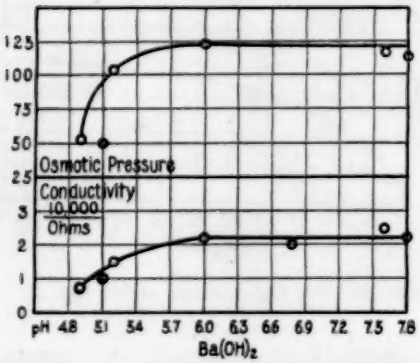



FIG. 7. See explanation under Fig. 2.

This means that in a 1 per cent solution of a metal gelatinate we have approximately three times as many particles in solution or suspension when the metal is univalent as when it is bivalent. Before we can draw any further conclusions we have to consider the relative conductivities of the same gelatin solutions.

A glance at the curves for conductivity shows that those for Na, Li, Ba, and Ca are almost identical, while the curves for K and $NH_4$ are a little higher than the others. These experiments were repeated and the same values were obtained. Fig. 8 shows that while the curves for conductivity of gelatin salts with univalent and bivalent metals (Na and Ba) are almost identical, the curves for the osmotic pressure of the two types of salts are very different.

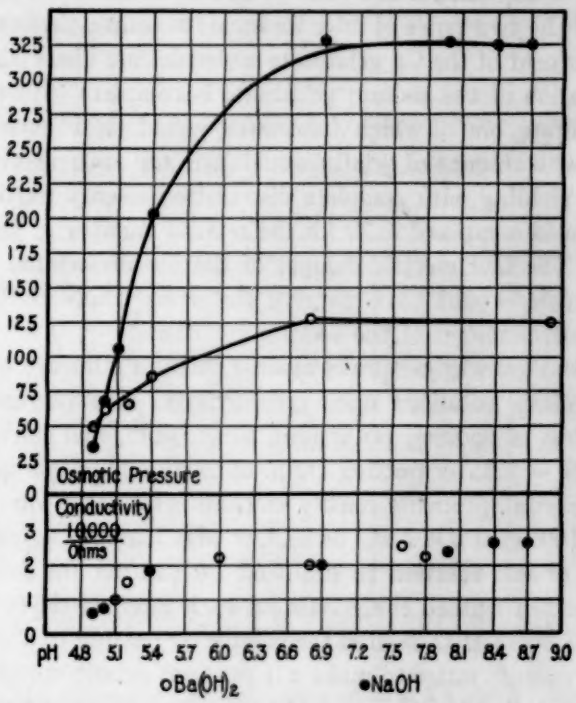



FIG. 8. Showing that while the curves for conductivity of sodium and barium gelatinate are practically identical, the curves for the osmotic pressures are very different.

There are two possible explanations for the fact that the ratio of conductivities of the two types of salts (Ca gelatinate and Na gelatinate) is 1:1 while the ratio of osmotic pressures is 1:3. The one explanation is that the degree of electrolytic dissociation of Ca gelatinate is so much smaller than that of Na gelatinate as to produce a

ratio of 1: 3 particles in solution. This would account for the difference in the ratio of osmotic pressures but would leave unexplained the identity of conductivity of the two solutions. It would therefore be necessary to make a second assumption; namely, that the lower viscosity of a 1 per cent Ca gelatinate solution would raise the conductivity of a Ca gelatinate solution enough to compensate for the smaller degree of electrolytic dissociation.

In order to account for the ratio of 1: 3 in osmotic pressure of the solutions of the two types of salts we have to assume that only about 20 to 25 per cent of the Ca gelatinate molecules are dissociated, while the dissociation of the sodium gelatinate is complete (five molecules of Ca gelatinate, one of which dissociates, would yield seven particles while the same amount of gelatin would form ten molecules of sodium gelatinate, yielding with complete dissociation twenty particles; this would result in a ratio of 7: 20 for the relative number of particles in solution). The four electric charges of the one dissociated Ca gelatinate molecule would have to give the same conductivity as the twenty electric charges of the sodium gelatinate.

Our present knowledge speaks against such an influence of the viscosity of gelatin solutions upon conductivity. We prepared 1 per cent solutions of sodium, potassium, magnesium, and calcium gelatinate, of pH = 7.0, by putting 1 gm. of finely pulverized commercial Cooper's gelatin (probably mostly calcium gelatinate) for 1 hour at 20°C. into 100 cc. of M/4 NaCl or KCl, or $MgCl_2$ or $CaCl_2$, and allowed the excess of salt solution to drain off by putting the gelatin on a filter. We then washed the gelatin on each filter six times in succession with 25 cc. of $H_2O$, melted the gelatin by heating to about 50°C., and added enough water to make a 1 per cent gelatin solution. The solution was cooled to 24°C. and the time of outflow through a viscometer, as well as the conductivity of each solution, was measured immediately, at 24°C. (Table I). We found the usual typical difference in viscosity between Ca and Mg gelatinate on the one hand, and Na and K gelatinate on the other. It is well known that the viscosity of a gelatin solution prepared by melting will increase on standing, especially at a low temperature. The gelatin solutions were kept at about 2°C. for 2 hours and were then heated to 24°, and their viscosity and conductivity were again measured. All the

viscosities had increased considerably and the viscosity of magnesium and calcium gelatinate was now as great as was originally that of the sodium gelatinate. Yet the conductivities were practically unaltered. The experiment was continued as indicated in Table I and enormous viscosities resulted, practically without any increase in the conductivities.

The reader will notice incidentally from the continuation of the experiment that upon heating to 50°C. and cooling to 24° the viscosity went practically back to its original level for the four different gela-

TABLE I.

*Variation of Viscosity and Conductivity of Gelatin Solutions upon Standing.*

*Viscosity in Seconds of Outflow, Conductivity,* $\frac{10{,}000}{Ohms}$.

*All Measurements at 24°C.*

| Treatment. | Na gelatinate. | | K gelatinate. | | Mg gelatinate. | | Ca gelatinate. | |
|---|---|---|---|---|---|---|---|---|
| | Viscosity. | Conductivity. | Viscosity. | Conductivity. | Viscosity. | Conductivity. | Viscosity. | Conductivity. |
| | *sec.* | $\frac{10{,}000}{ohms}$ | *sec.* | $\frac{10{,}000}{ohms}$ | *sec.* | $\frac{10{,}000}{ohms}$ | *sec.* | $\frac{10{,}000}{ohms}$ |
| Immediately after melting... | 99 | 2.31 | 102 | 3.07 | 88 | 2.10 | 86 | 2.12 |
| After 1 hr. in refrigerator and heating to 24°C..... | 135 | 2.22 | 143 | 2.94 | 138 | 2.06 | 130 | 2.12 |
| After 18 hrs. in refrigerator and heating to 24°C..... | 180 | 2.28 | 250 | 3.03 | 240 | 2.07 | 200 | 2.09 |
| After being kept at 24° for 2 hrs..... | 170 | 2.35 | 210 | 3.09 | 194 | 2.10 | 173 | 2.13 |
| After heating to 50° and cooling to 24°..... | 94.5 | 2.31 | 95.5 | 3.11 | 86 | 2.12 | 83.5 | 2.14 |

tin salts. In this case the influence of the "history" upon the colloidal solution is entirely reversible.

These experiments seem to exclude the assumption that the degree of electrolytic dissociation of calcium gelatinate is so much smaller than that of sodium gelatinate that it produces a ratio of 1:3 in the osmotic pressure of the two solutions; and that the difference in the degree of dissociation is compensated by the influence of viscosity upon conductivity in such a way as to make the conductivities of the solution of the two types of salts equal.

The second possible explanation is based on the assumption that the equality of conductivity is due to the fact that both types of solutions in equal concentration and for the same pH possess an approximately equal number of charges.

The identity of equivalents combining with gelatin demands that twice as many gelatin molecules must combine with one atom of Ca as with one atom of Na. For the sake of simplification we assume that one Na atom combines with one gelatin atom. This would mean that calcium gelatinate exists in the form of Ca gelatin$_2$ or Ca$_2$ gelatin$_4$ or Ca$_3$ gelatin$_6$, and sodium gelatinate in the form of Na gelatin. In this case all phenomena will find their explanation if we assume that in the dissociation of Ca$_2$ gelatin$_4$ the four gelatin ions remain aggregated in one group with four negative charges

$$Ca_2\,gelatin_4 \rightleftharpoons Ca^{++} + Ca^{++} + [gelatin]_4^{----}$$

Such a dissociation would therefore yield three ions, one of which contains an aggregate of four negative gelatin ions. In order to obtain the same number of charges, four molecules of Na gelatin would be required, dissociating into four positive Na ions and four separate negative gelatin ions, making eight ions in all. This would demand a ratio of osmotic pressures for the two gelatin solutions of 3:8, which is slightly less than the ratio observed. The electrical charges would be the same for the two solutions and the conductivities would only show the difference due to differences in the ionic mobilities.

If the dissociating complex in the case of calcium gelatinate is Ca$_3$ gelatin$_6$ resulting in the formation of three Ca ions and one aggregate gelatin$_6$ anion carrying six charges, the same number of charges would be carried by six molecules of sodium gelatinate dissociating into twelve ions. This would yield exactly the ratio of 1:3 for the osmotic pressure of solutions of calcium gelatinate and sodium gelatinate of the same concentration and conductivity.

If the aggregates consist of eight gelatin anions with four Ca ions the ratio of osmotic pressures would be 5:16 which is also approximately 1:3.

It is in reality only necessary to assume the existence of compounds of the form Ca gelatin$_2$, the two anions of which form one aggregate of two gelatin anions, and to assume further that two, three,

or more such aggregates of two gelatin anions join to form larger aggregates of four, six, or eight gelatin anions, every one of which keeps its original charge. This would account for all the phenomena observed.

We are only able to estimate the relative difference in the mobilities of the cations. They are higher for K and $NH_4$ than for Na and Li, and we also find that the observed conductivities of K and $NH_4$ gelatinate in our experiments are higher than those of Na and Li gelatinate, possibly to the amount the difference in mobility of the ions named demands.

Bayliss in comparing the osmotic pressure and the electrolytic dissociation of solutions of Congo red found that it is ionized 80 per cent in a dilution of 500 liters, yet,

> "The osmotic pressure [of such solutions] found experimentally, both by direct measurement and by vapour pressure, is, throughout a wide range of concentration, uniformly between 95 and 100 per cent. of what it would be if no dissociation existed. Since it should be from one and a half to three times this value, according to the concentration, it is plain that there are some abnormal conditions present."

Bayliss suggests an explanation similar to the one given above; namely, "the possibility of aggregated simple ions carrying the sum of the charges of their components."[8]

### *IV. Influence of Valency upon Swelling and Viscosity.*

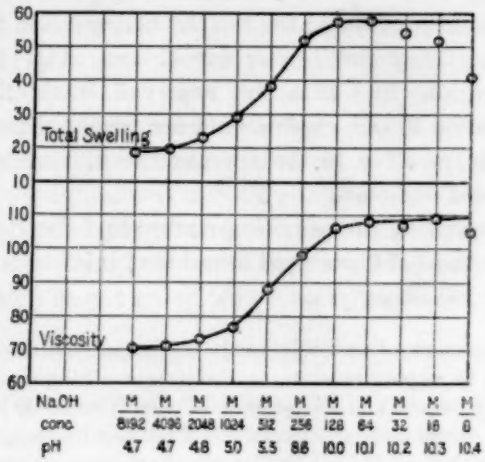
It has been demonstrated in previous papers that the curves for the values of viscosity and swelling are similar to the curves for osmotic pressure and it has been stated that these properties must also be a function of the relative number of metal gelatinate molecules and ions formed.

In Figs. 9 and 10 we give the curves for viscosity and swelling of sodium and potassium gelatinate and in Figs. 11 and 12 the same curves for barium gelatinate and calcium gelatinate. The values for viscosity are given in times of outflow and the values for swelling in terms of the height of cylinders of gelatin of the same diameter.[9] On

[8] Bayliss, W. M., *Proc. Roy. Soc. London, Series B*, 1911, lxxxiv, 253, 254.

[9] Loeb, J., *J. Biol. Chem.*, 1918, xxxiv, 77, 395.

the axis of abscissæ are the logarithms of the concentrations of the alkali solution used. The pH of the gelatin is given under each concentration. The values for Na and K gelatinate are practically iden-




FIGS. 9 to 12. Curves for viscosity and swelling of sodium gelatinate (Fig. 9), potassium gelatinate (Fig. 10), calcium gelatinate (Fig. 11), and barium gelatinate (Fig. 12). The curves for sodium gelatinate differ from the curves for Ca and Ba gelatinate in the same sense and almost the same degree as the curves for osmotic pressure.

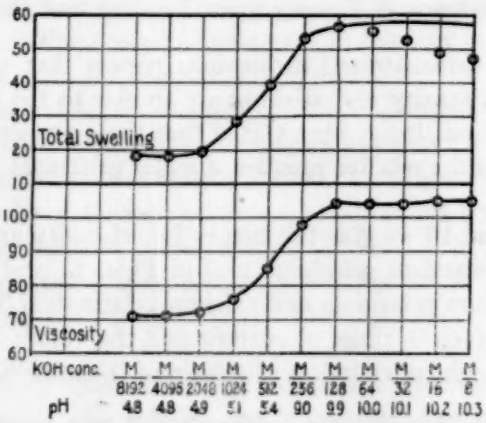



FIG. 10. See explanation under Fig. 9.

tical, and so are the values for Ca and Ba gelatinate. But the values for Na gelatinate and Ca gelatinate differ. The reader will notice that the values for pH are higher than in the curves for osmotic pressure. This is due to the fact that in the latter case the pH was determined after the excess of alkali had been removed by washing and dialysis, while the nature of the experiment made it necessary for us

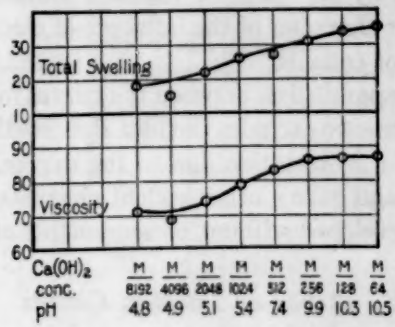



FIG. 11. See explanation under Fig. 9.

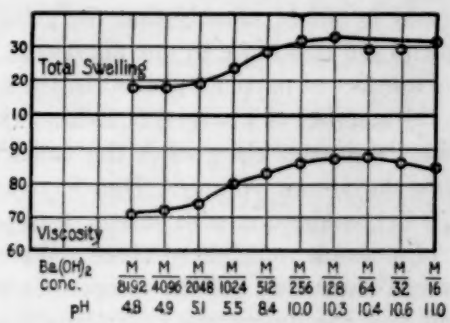



FIG. 12. See explanation under Fig. 9.

to determine the pH in the experiments on swelling and viscosity only after washing away the greater part of the excess of alkali, without using dialysis.

Neither the values for swelling nor for viscosity are as accurate as those for the osmotic pressure and hence the former cannot well serve for the purpose of drawing conclusions in regard to the molecular

basis of these properties. The viscosity rises for some time after the melting of gelatin and only approximately constant values can be obtained by measuring the time of outflow immediately; *i.e.*, within 5 minutes after the melting. The swelling is influenced by the resistance of the gelatin to the expanding forces leaving aside other sources of error. The osmotic pressure in connection with measurements on conductivity seems by far the most valuable property for the analysis of the molecular character of the influence of electrolytes upon the physical behavior of colloids.

The approximate parallelism between the curves for viscosity, swelling, and osmotic pressure excludes the idea that swelling and viscosity are determined by a hydratation due to the degree of ionization of proteins as Pauli and many other colloid chemists assume. These hypotheses were developed without measurements of conductivity.

### *V. Action of Trivalent Cations.*

The facts of the preceding chapters furnish a suggestion for the understanding of the action of trivalent metals. If the same tentative assumption may be made, namely that the gelatin ions held by the polyvalent metal ion dissociate in one aggregate, thus causing a diminution in the number of particles present in the solution without a diminution in the number of charges, it follows that this anionic aggregate must be the greater the greater the valency of the metal ion—leaving aside the atomic volume. Hence trivalent metals like Ce or Al should, if in combination with gelatin, lead to anionic aggregates consisting of a simple multiple of three. We cannot test this inference but we can show that gelatin compounds with a trivalent metal like cerium and aluminium are practically insoluble, and this insolubility might be the consequence of the increase in the number of gelatin anions forming an aggregate with the valency of cations.

1 gm. of finely powdered Cooper's gelatin (which is essentially calcium gelatinate of pH = 7.0) was put for 1 hour into each of a series of beakers containing 100 cc. of a solution of $Ce_2Cl_6$ varying in concentration from M/4 to M/16,384, then drained on a filter and washed four times with distilled water to remove the excess of salt solution. The conductivity osmotic pressure. alcohol number, and total swell-

ing were ascertained. The results are plotted in Fig. 13. Gelatin treated with $Ce_2Cl_6$ solution from M/4 to M/2048 becomes insoluble and the cerium gelatinate forms a precipitate. The values for conductivity, etc., were as low as those obtained for gelatin at the isoelectric point. The gelatin treated with M/4096 or less concentrated $Ce_2Cl_6$ was clear and gave values for conductivity, etc., rising

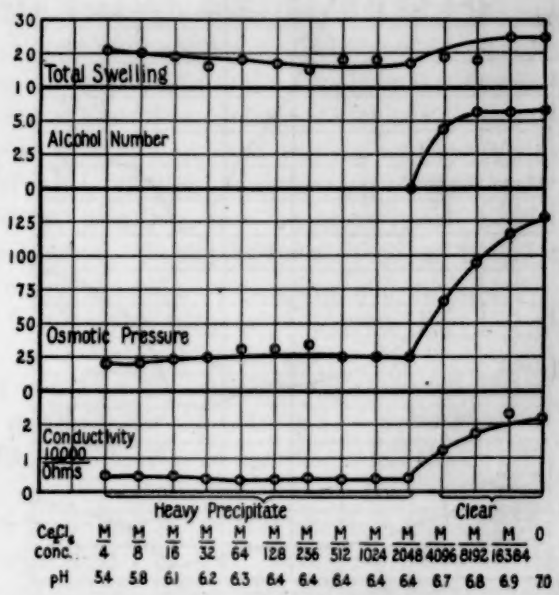



FIG. 13. Impure gelatin (chiefly calcium gelatinate) of pH 7.0 treated with concentrations of $Ce_2Cl_6$ varying from M/4 to M/16,384, then freed from excess of salts. pH of the gelatin solution is given under each solution. Cerium gelatinate is insoluble and hence the values for swelling, alcohol number, osmotic pressure, and conductivity are almost zero except when the concentration of the $Ce_2Cl_6$ used was low (M/4096 and less), when only a fraction of the original calcium gelatinate was transformed into cerium gelatinate.

with increasing solutions of $Ce_2Cl_6$. In these cases not all the Ca gelatinate was transformed into Ce gelatinate. The less Ce gelatinate was formed the higher the value for conductivity, osmotic pressure, alcohol number, and swelling. The pH was between 5.4 and 7.0; *i.e.*, on the alkaline side of the isoelectric point of gelatin. Lead and copper acetates gave a similar result, as was to be expected.

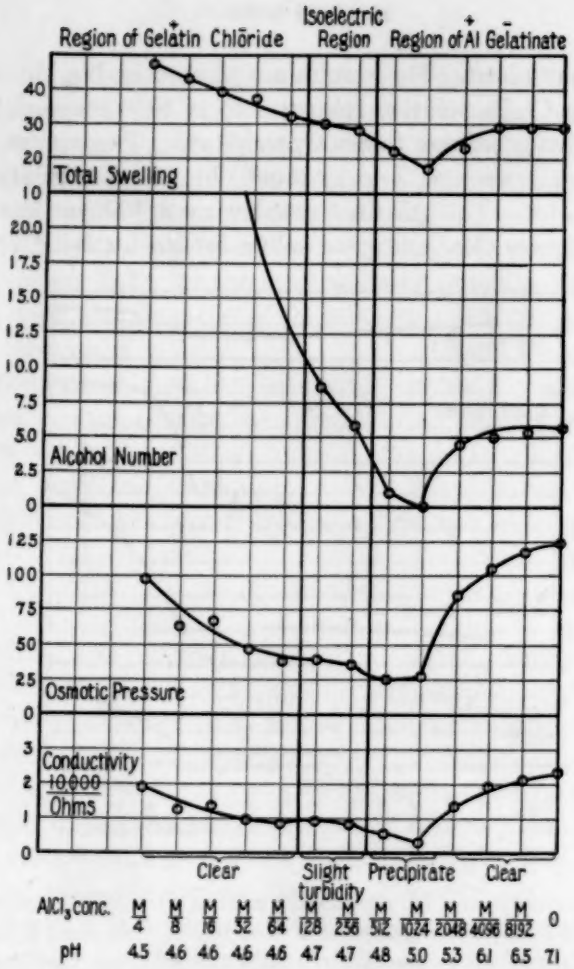



FIG. 14. Impure gelatin (chiefly calcium gelatinate) treated with different concentrations of an acid salt with trivalent cation, namely, $AlCl_3$. As long as pH $< 4.7$ no aluminium gelatinate is formed and the gelatin exists in the form of gelatin chloride which gives a clear solution. At the isoelectric region pH $= 4.7$ gelatin is insoluble and all the curves show the low level characteristic of gelatin at the isoelectric point. When pH $> 4.7$ gelatin exists as Al gelatinate. As long as the concentration of $AlCl_3$ used was high enough to transform all the original calcium gelatinate into aluminium gelatinate, M/512 to M/1024, all the gelatin was transformed into the insoluble aluminium gelatinate which formed a precipitate as in the case of cerium gelatinate. When the concentration of the $AlCl_3$ solution used was less than M/1024 not all the calcium gelatinate was transformed into aluminium gelatinate and the solution became gradually clear again.

$AlCl_3$ forms acid solutions and it seemed of interest to study the effect of a treatment of gelatin with $AlCl_3$. We know now that for pH <4.7 gelatin treated with $AlCl_3$ must form gelatin chloride and cannot be affected by the aluminium. The same is true also for pH = 4.7, where gelatin exists as sparingly soluble pure gelatin. For pH > 4.7 aluminium gelatinate can be formed which should be insoluble. If, however, the concentration of $AlCl_3$, becomes too low, less and finally practically no aluminium gelatinate should be formed. These predictions were all fulfilled as the complicated curves in Fig. 14 show. 1 gm. of Cooper's powdered gelatin (*i.e.* chiefly Ca gelatinate) was put for 1 hour at 20° into a series of beakers, each containing 100 cc. of a solution of $AlCl_3$ varying in concentration from M/4 to M/8192. The gelatin was then freed from the excess of salt solution by washing as described before. In Fig. 14 the abscissæ are the logarithms of the concentration of the $AlCl_3$ solution used and the final pH of the washed gelatin is found under each concentration. For pH <4.7 no effect of aluminium is noticeable; the curves are typical gelatin chloride curves. For pH = 4.7 we find a region where gelatin behaves like isoelectric gelatin. For pH = 4.8 and 5.0 we have a precipitate formation due to the formation of aluminium gelatinate. When the concentration of $AlCl_3$ solution used is less than M/1024 the solution becomes clear again and the curves rise with increasing dilution of the $AlCl_3$ solution used. In other words, for pH >4.7 the curves for aluminium gelatinate and cerium gelatinate run parallel as our theory demands.[10]

This curve suggests incidentally why in the writer's former experiments on *Fundulus* eggs the antagonistic influence of $AlCl_3$ to the toxic action of a sodium chloride solution was so restricted. On the acid side of the isoelectric point of amphoteric colloids $AlCl_3$ cannot act on the colloid, and on the basic side the concentration of Al is too low. It is the writer's intention to repeat these experiments with $Ce_2Cl_6$ or $LaCl_3$, the solutions of which are less acid.

[10] One wonders what interpretation such a curve as that for $AlCl_3$ would have found on the part of the colloid chemists who have persistently ignored not only the hydrogen ion concentration of their protein solutions but who have also overlooked the chemical significance of the isoelectric point.

SUMMARY.

1. A method is given by which the amount of equivalents of metal in combination with 1 gm. of a 1 per cent gelatin solution previously treated with an alkali can be ascertained when the excess of alkali is washed away and the pH is determined. The curves of metal equivalent in combination with 1 gm. of gelatin previously treated with different concentrations of LiOH, NaOH, KOH, $NH_4OH$, $Ca(OH)_2$, and $Ba(OH)_2$ were ascertained and plotted as ordinates, with the pH of the solution as abscissæ, and were found to be identical. This proves that twice as many univalent as bivalent cations combine with the same mass of gelatin, as was to be expected.

2. The osmotic pressure of 1 per cent solutions of metal gelatinates with univalent and bivalent cation was measured. The curves for the osmotic pressure of 1 per cent solution of gelatin salts of Li, Na, K, and $NH_4$ were found to be identical when plotted for pH as abscissæ, tending towards the same maximum of a pressure of about 325 mm. of the gelatin solution (for pH about 7.9). The corresponding curves for Ca and Ba gelatinate were also found to be identical but different from the preceding ones, tending towards a maximum pressure of about 125 mm. for pH about 7.0 or above. The ratio of maxi mal osmotic pressure for the two groups of gelatin salts is therefore about as 1: 3 after the necessary corrections have been made.

3. When the conductivities of these solutions are plotted as ordinates against the pH as abscissæ, the curves for the conductivities of Li, Na, Ca, and Ba gelatinate are almost identical (for the same pH), while the curves for the conductivities of K and $NH_4$ gelatinate are only little higher.

4. The curves for the viscosity and swelling of Ba (or Ca) and Na gelatinate are approximately parallel to those for osmotic pressure.

5. The practical identity or close proximity of the conductivities of metal gelatinates with univalent and bivalent metal excludes the possibility that the differences observed in the osmotic pressure, viscosity, and swelling between metal gelatinates with univalent and bivalent metal are determined by differences in the degree of ionization (and a possible hydratation of the protein ions).

6. Another, as yet tentative, explanation is suggested.